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Growth of Lactobacillus acidophilus ATCC 4357 on different substrates 11/30/00.

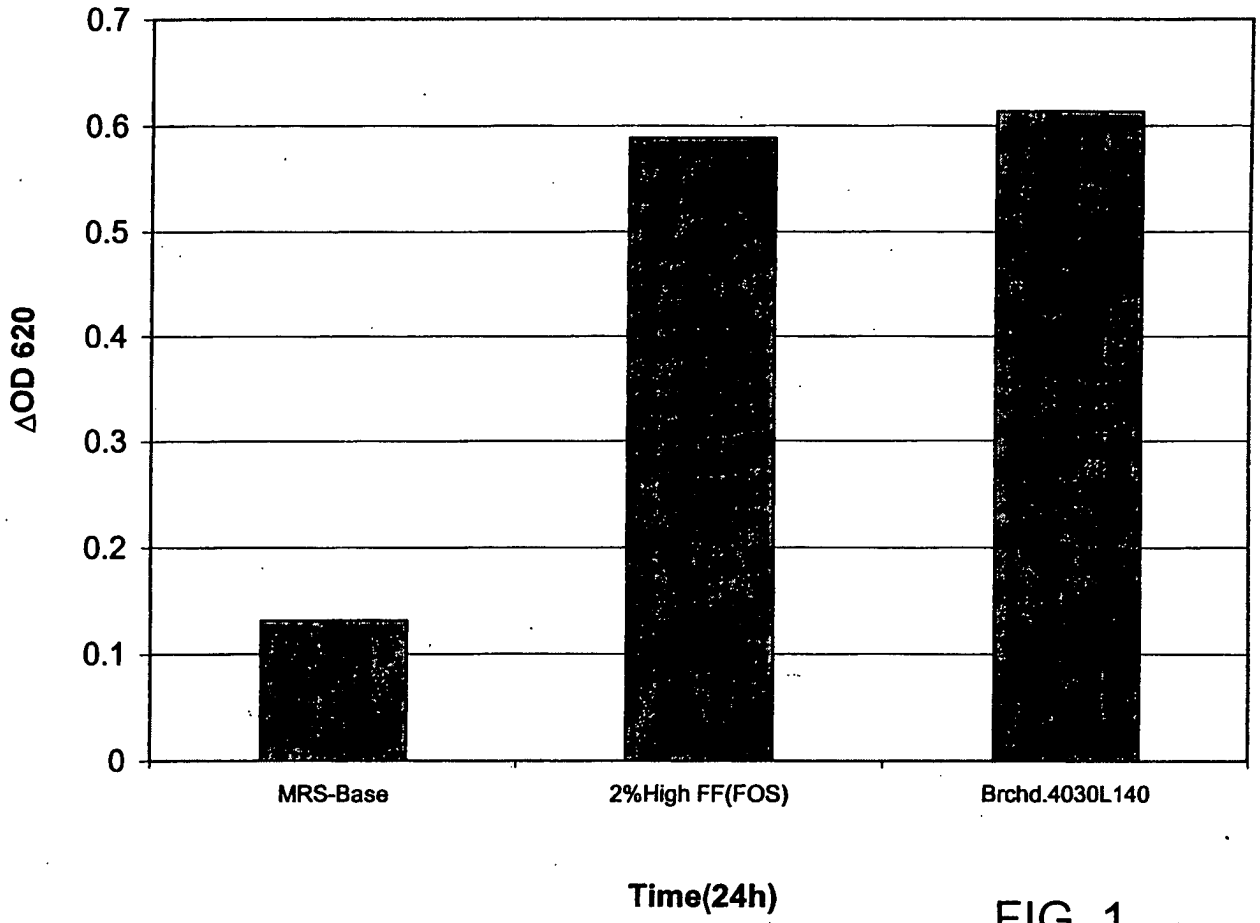


FIG. 1

Growth of Lactobacillus johnsonii ATCC 33200 on different substrates

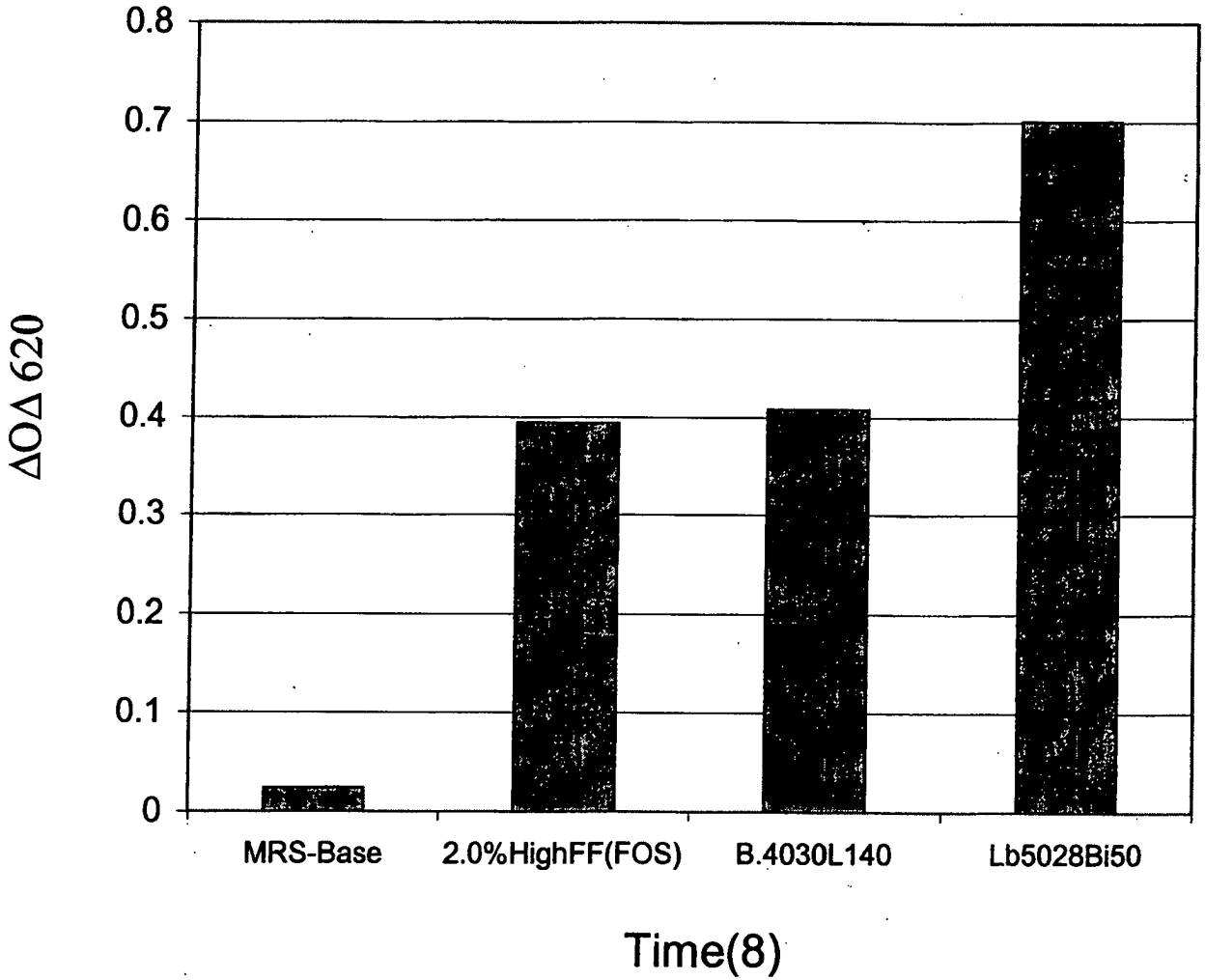


FIG. 2

Growth of Lactobacillus amylovorus ATCC 33620 on different substrates 12/8/00.

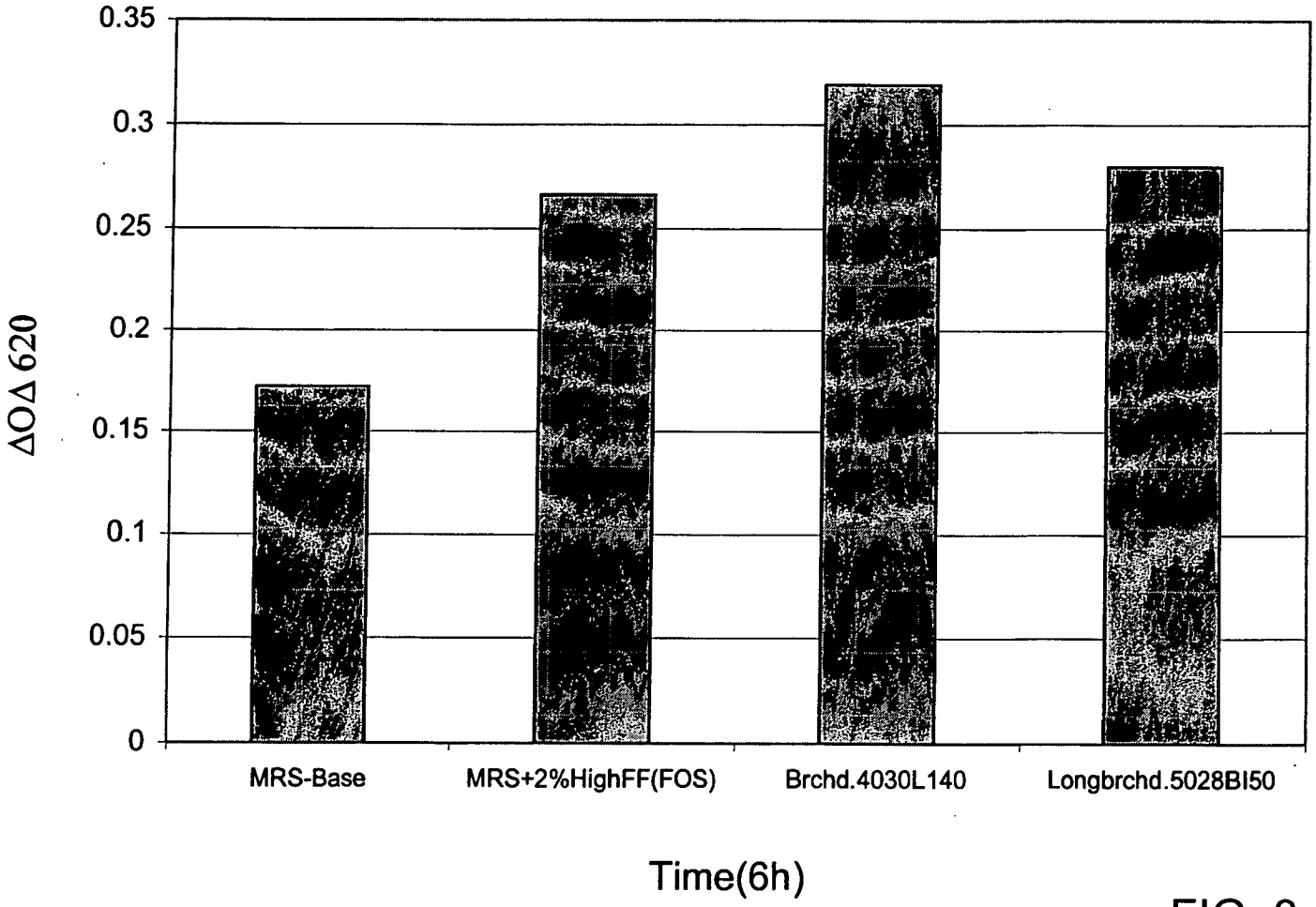


FIG. 3

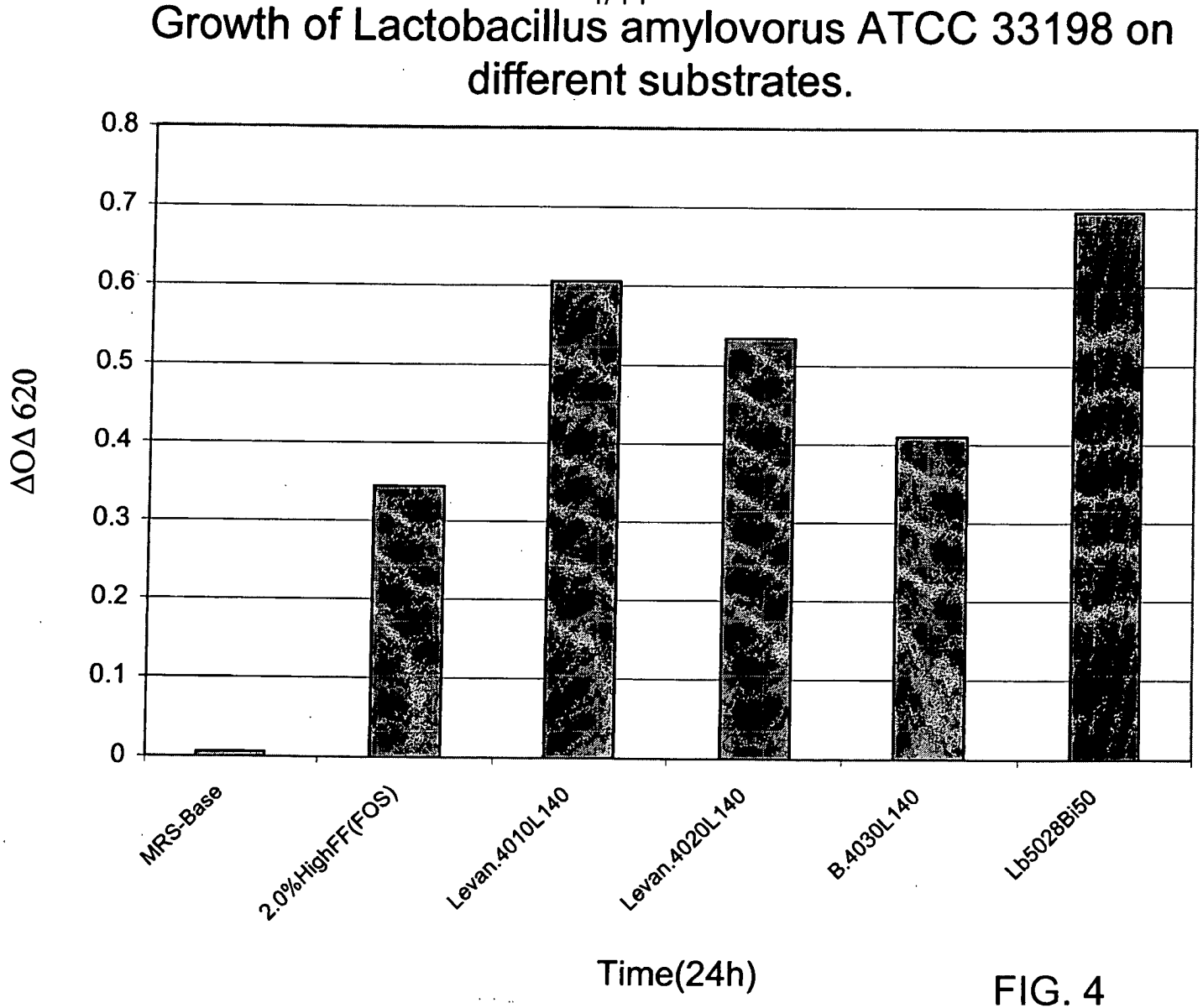

Growth of Lactobacillus amylovorus ATCC 33198 on different substrates.
ΔOΔ 620
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
MRS-Base
2.0%HighFF(FOS)
Levan.4010L140
Levan.4020L140
B.4030L140
Lb5028Bi50
Time(24h)

FIG. 4

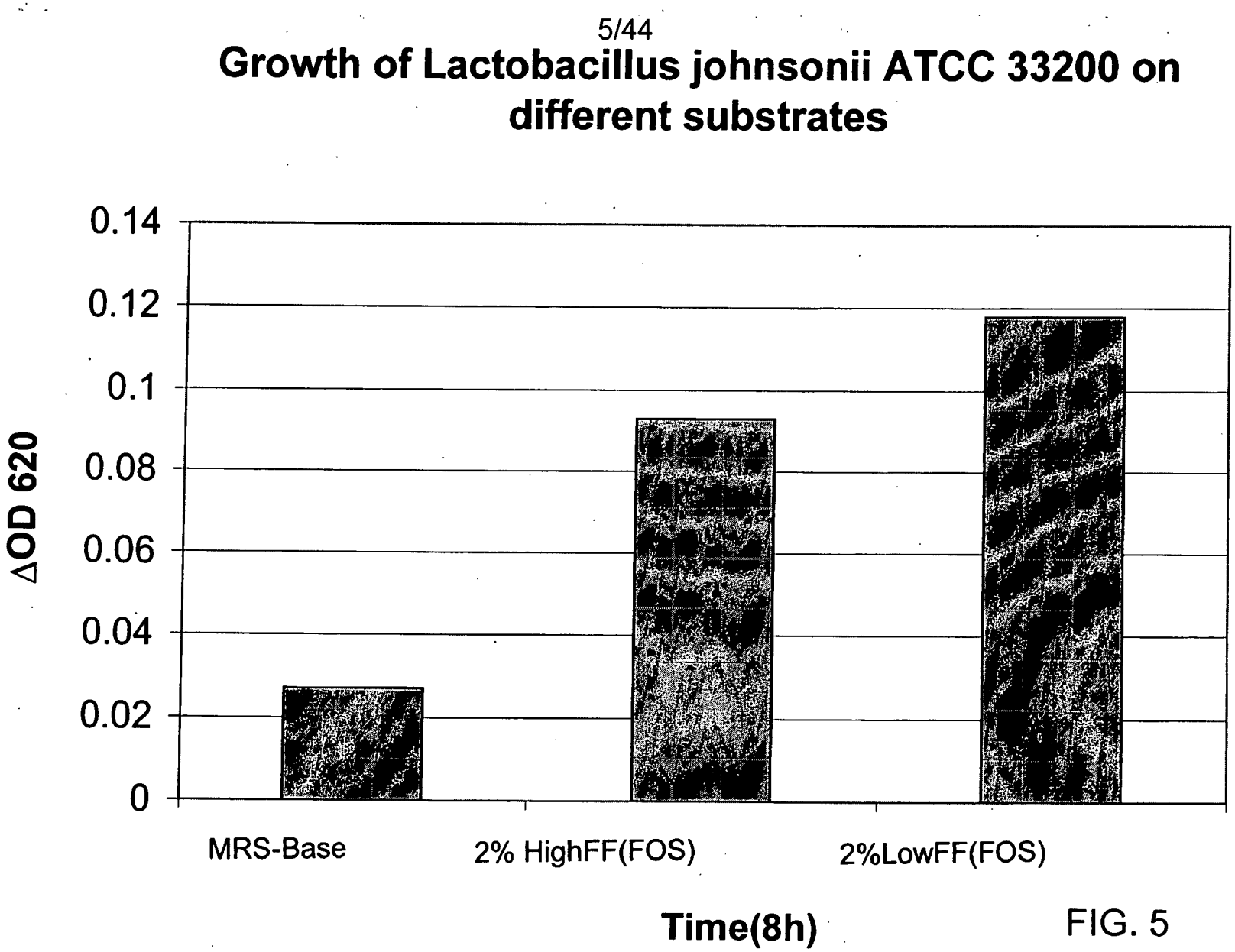

Growth of Lactobacillus johnsonii ATCC 33200 on different substrates
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
ΔOD 620
MRS-Base
2% HighFF(FOS)
2%LowFF(FOS)
Time(8h)

FIG. 5

Growth of Lactobacillus plantarum ATCC 4008 on different substrates 5/23/01.

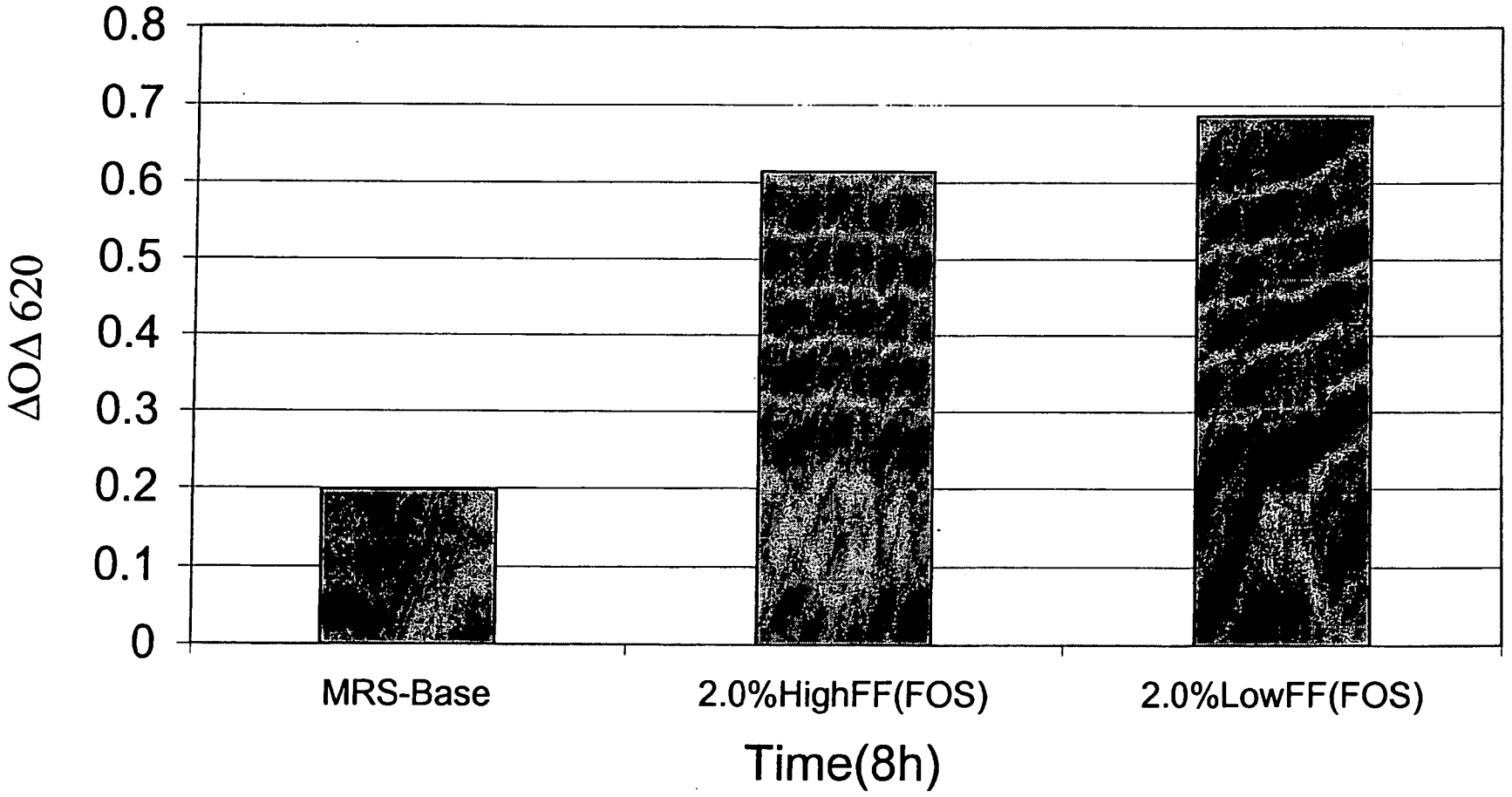


FIG. 6

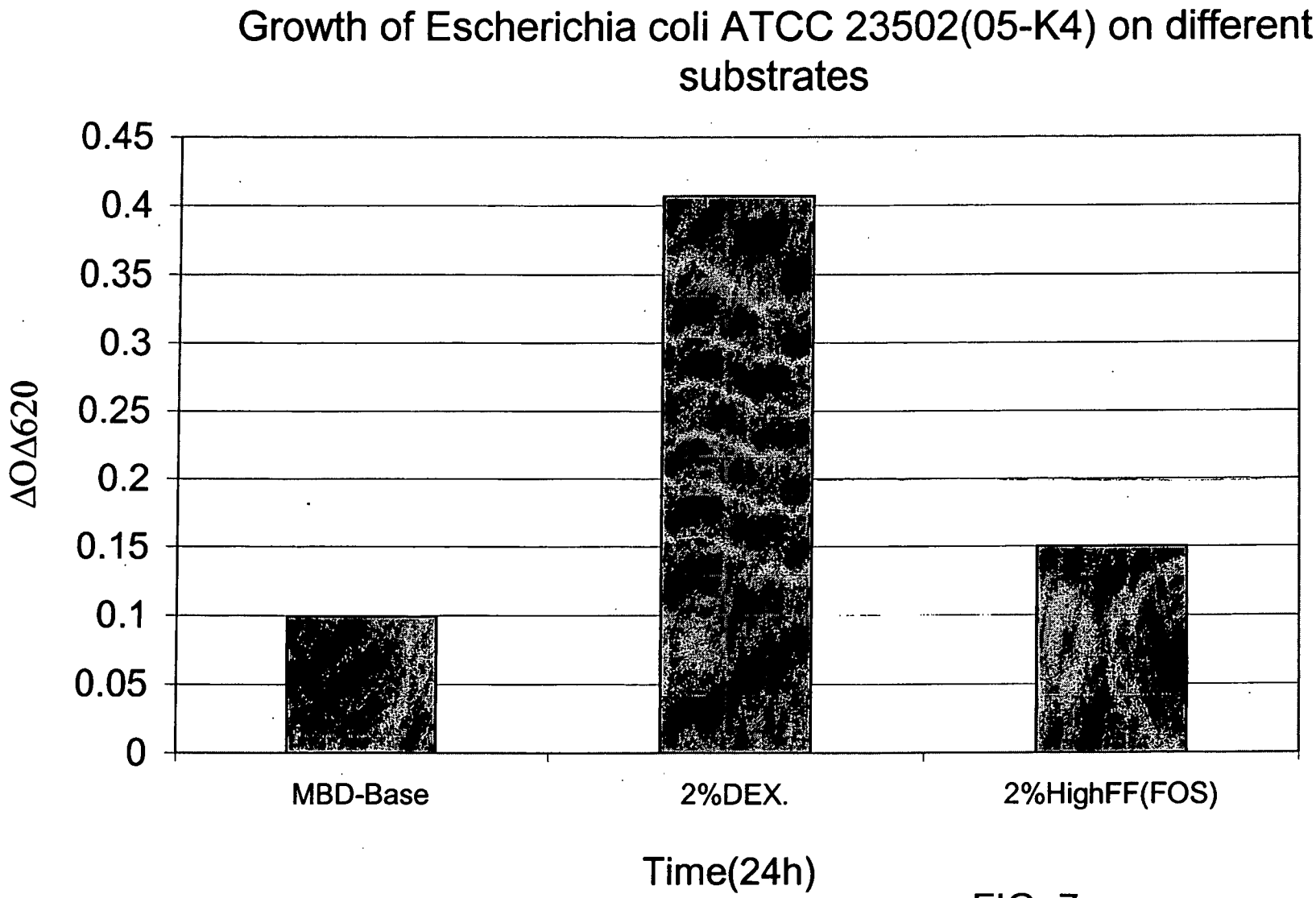

Growth of Escherichia coli ATCC 23502(05-K4) on different substrates
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
ΔOΔ620
MBD-Base
2%DEX.
2%HighFF(FOS)
Time(24h)

FIG. 7

Growth of Escherichia coli U1-41(05-K4) on different substates 1/4/01.

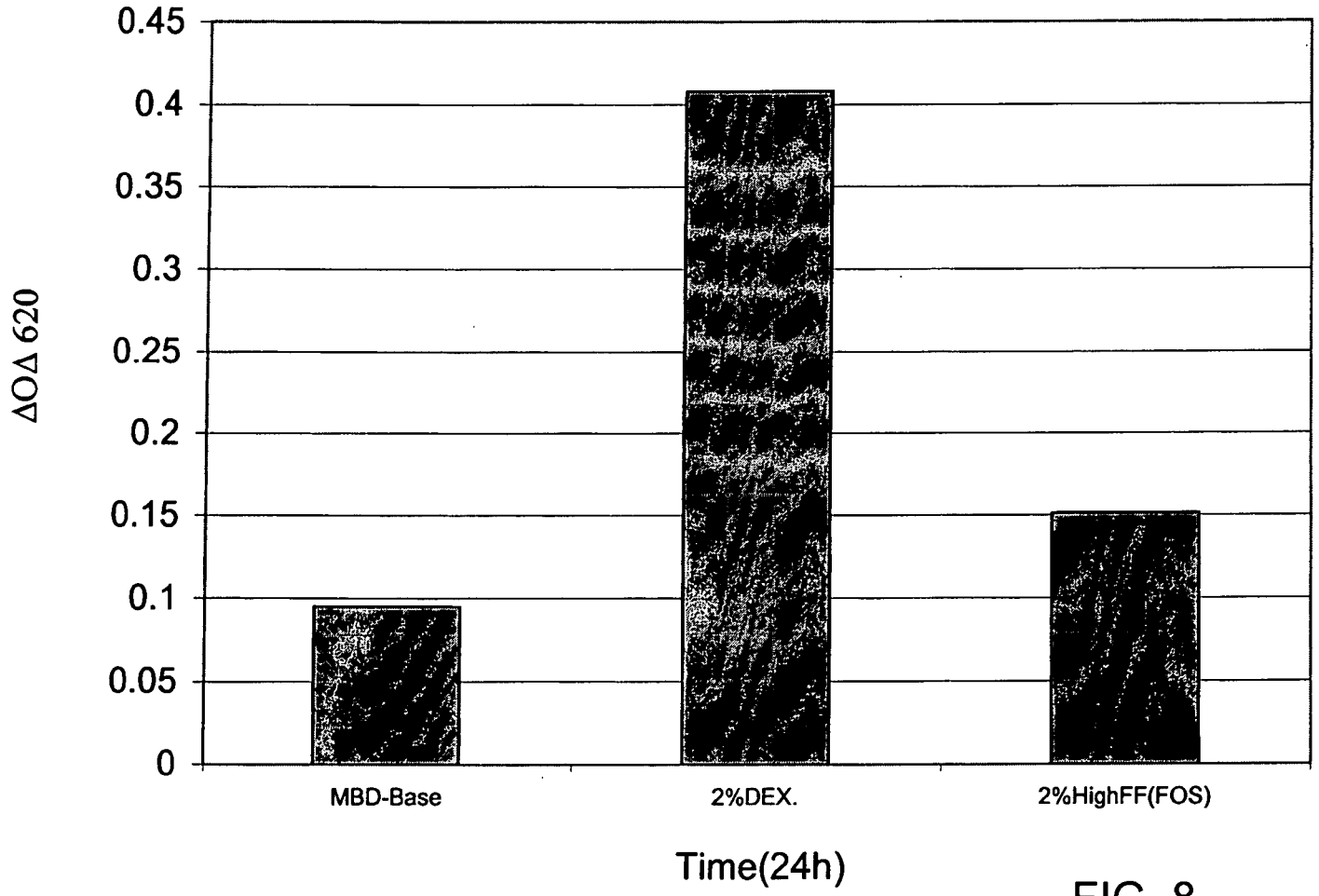


FIG. 8

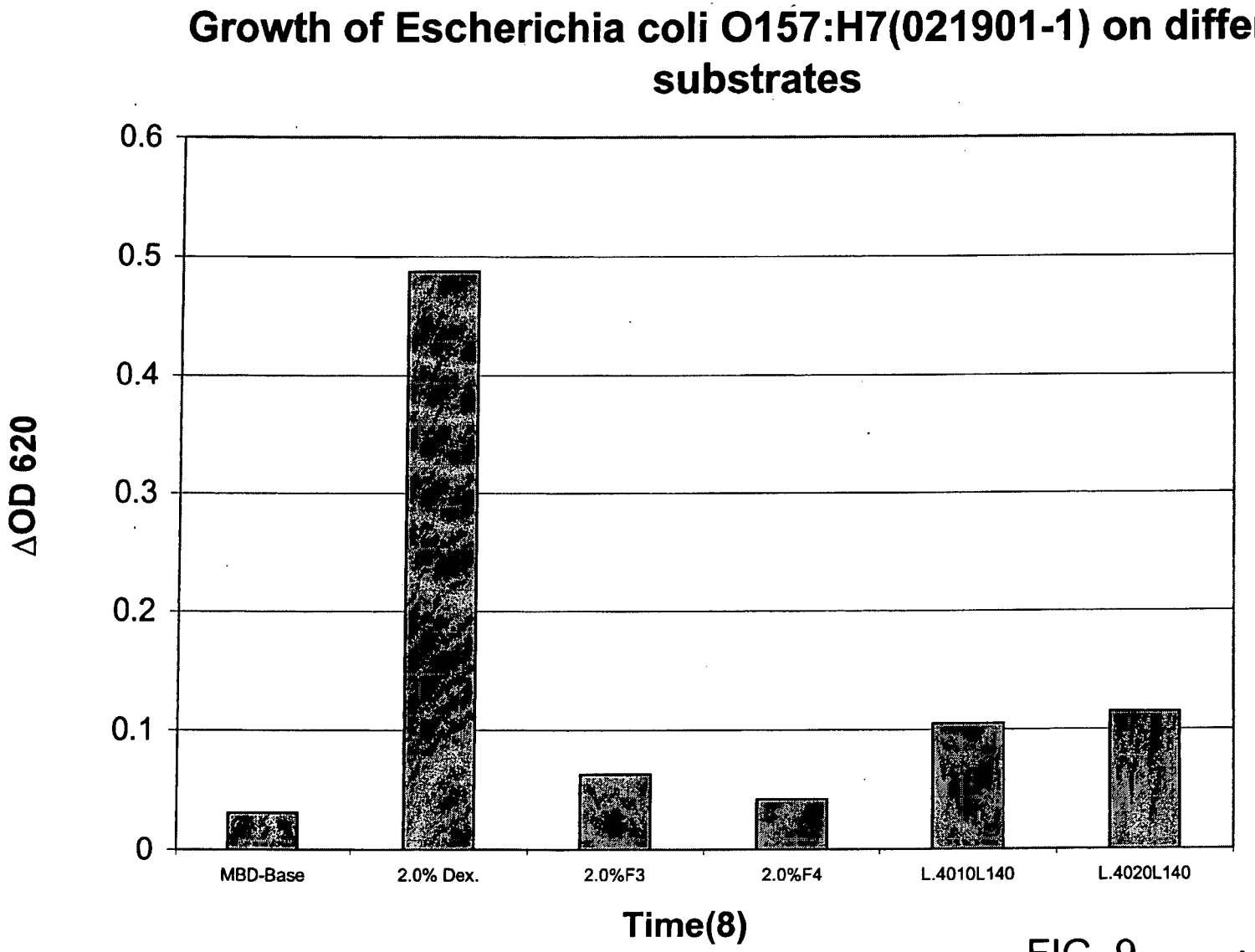

Growth of Escherichia coli O157:H7(021901-1) on different substrates
0.6
0.5
0.4
0.3
0.2
0.1
0
ΔOD 620
MBD-Base
2.0% Dex.
2.0%F3
2.0%F4
L.4010L140
L.4020L140
Time(8)

FIG. 9

Growth of Salmonella group B (021901-5) on different substrates 3/9/01.

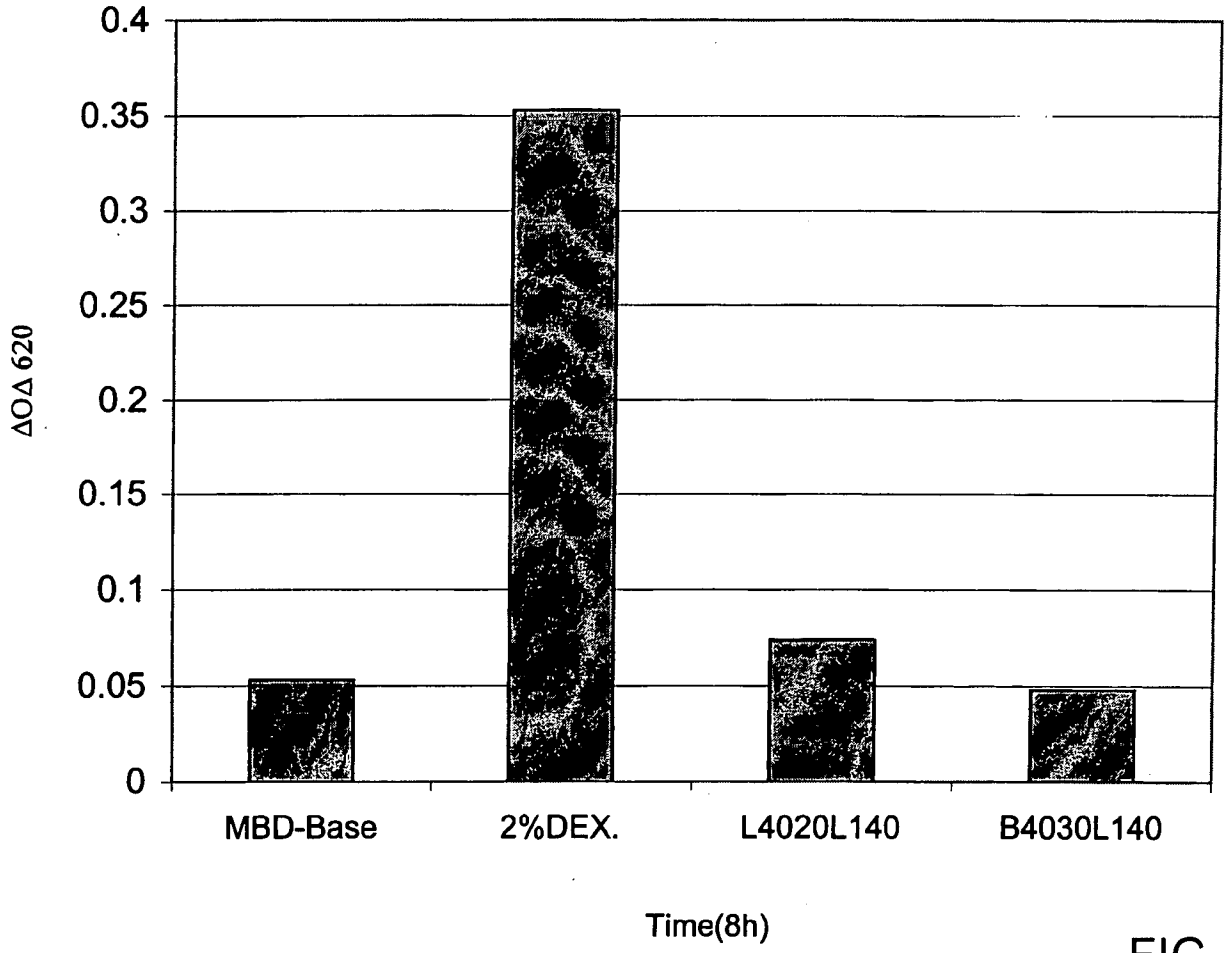


FIG. 10

Levan Distribution - Single Trapping Experiment Versus Previous Averages

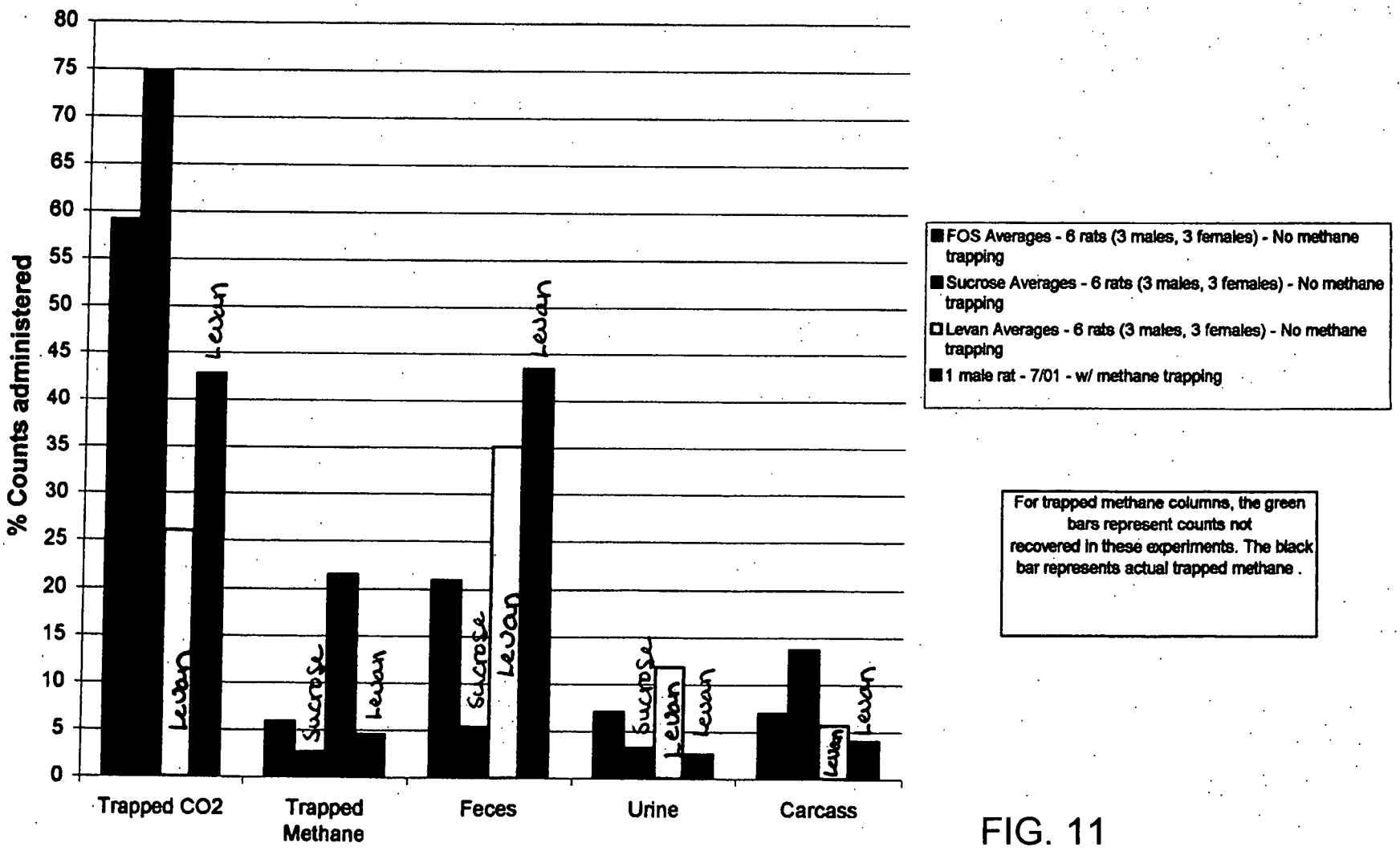


FIG. 11

5

Levan 4010LI40 @50 oC

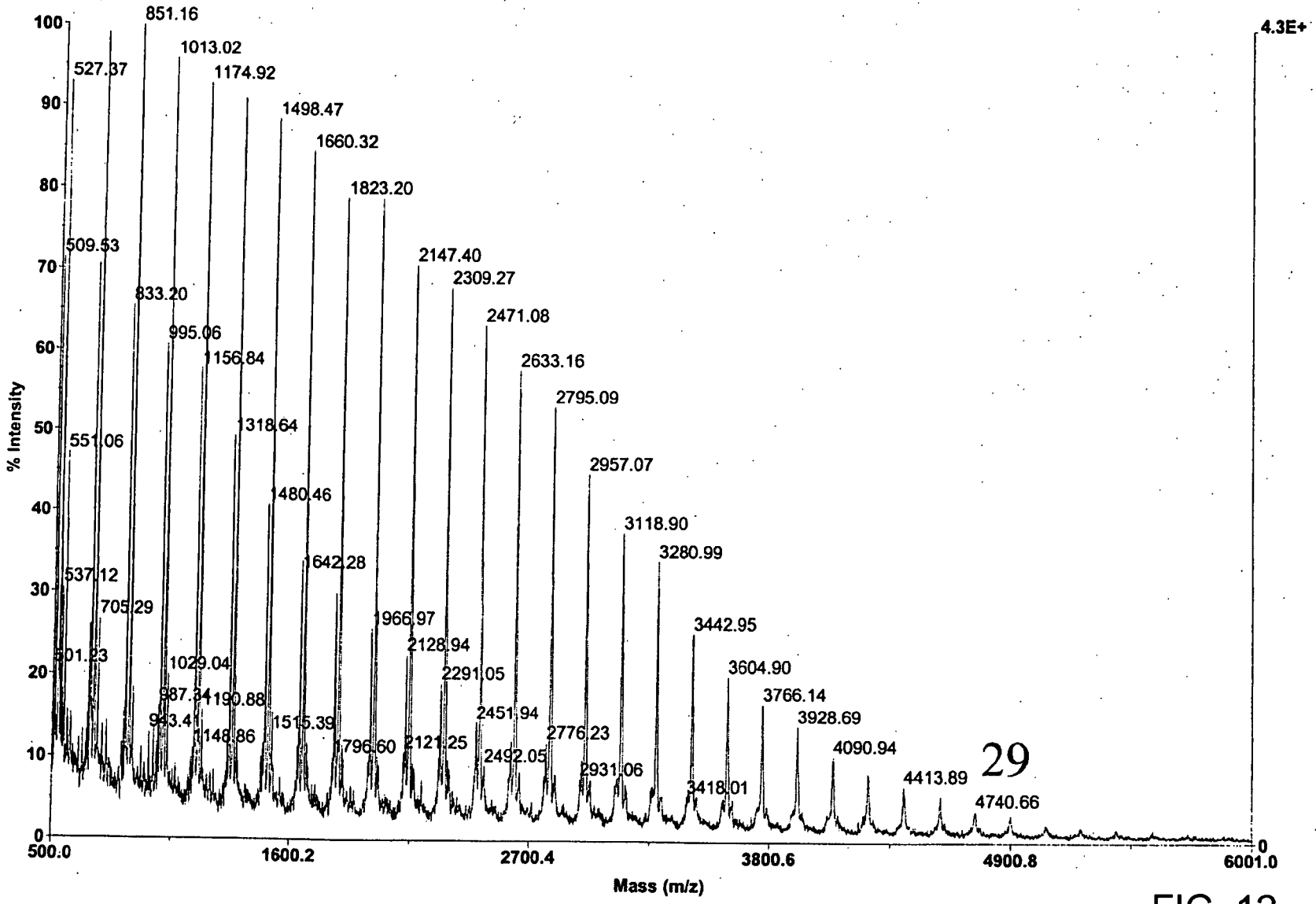


FIG. 12

Short-branched fructan 4030LI40 @ 2:1 FOS:Sucrose

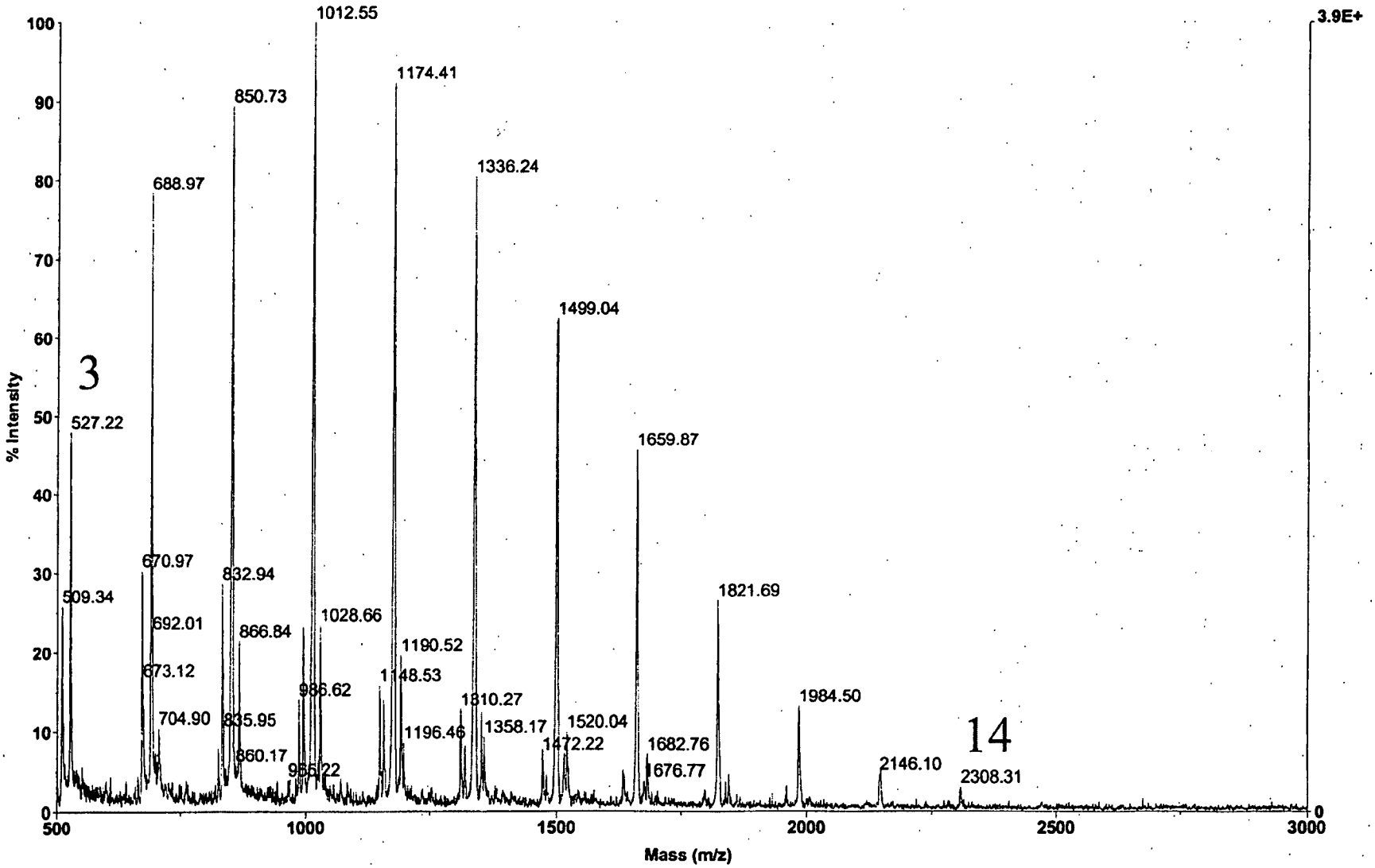


FIG. 13

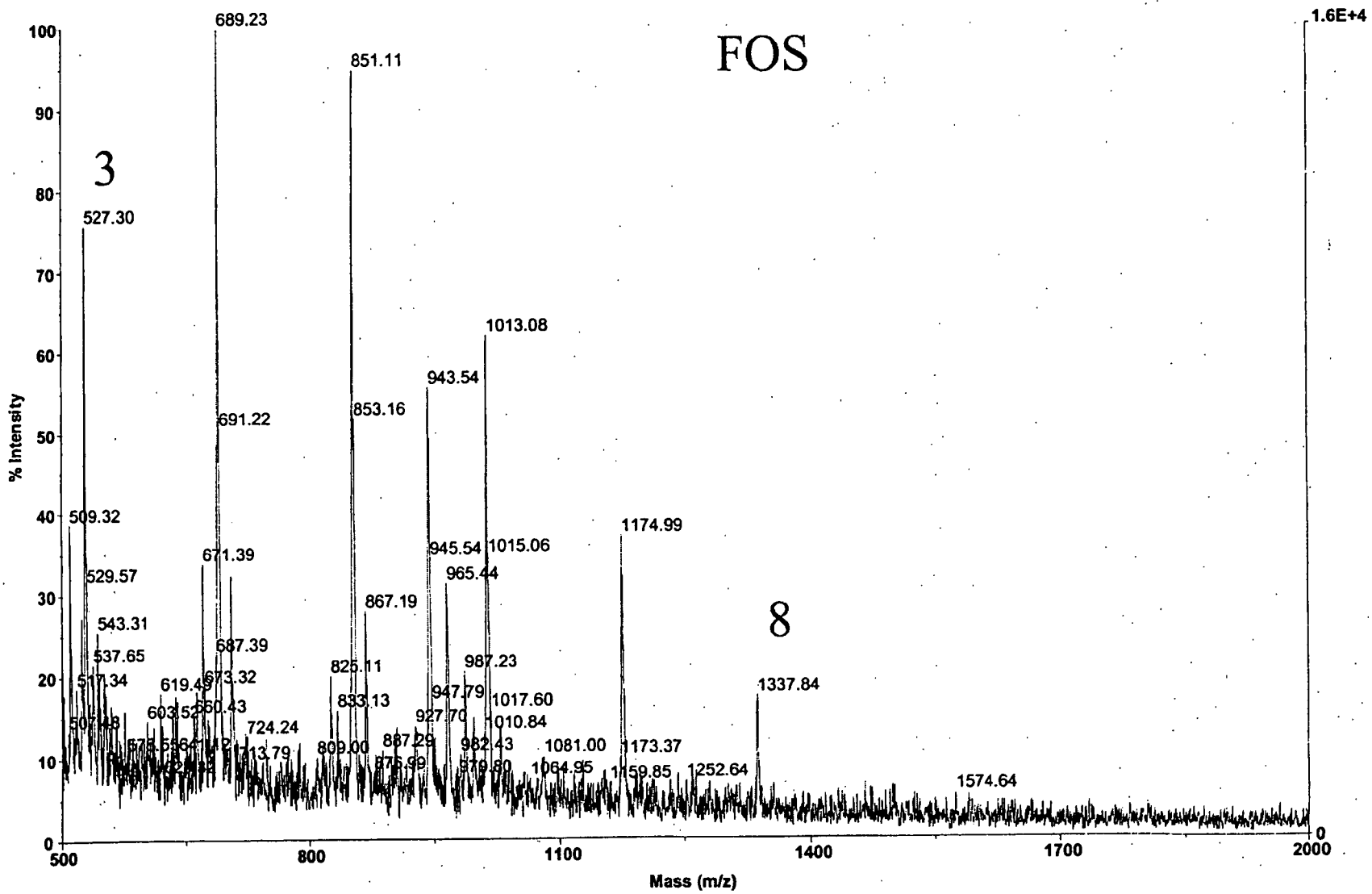

FOS
3
8
% Intensity
Mass (m/z)
1.6E+4
0
689.23
851.11
527.30
1013.08
943.54
691.22
853.16
1174.99
509.32
671.39
945.54
1015.06
965.44
529.57
867.19
543.31
687.39
537.65
825.11
987.23
673.32
619.49
947.79
1337.84
833.13
1017.60
603.52
560.43
927.70
1010.84
724.24
809.00
887.29
982.43
1081.00
1173.37
1064.95
1159.85
1252.64
1574.64

FIG. 14

FIG. 15A

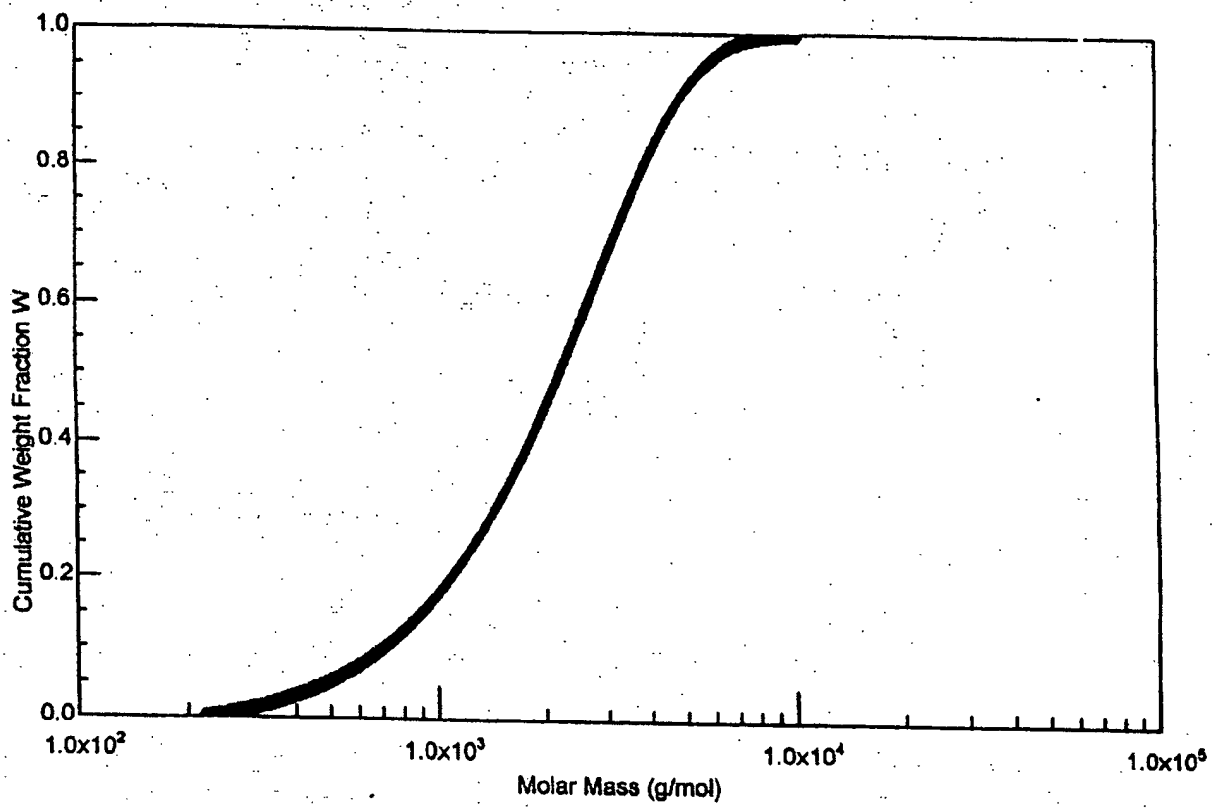
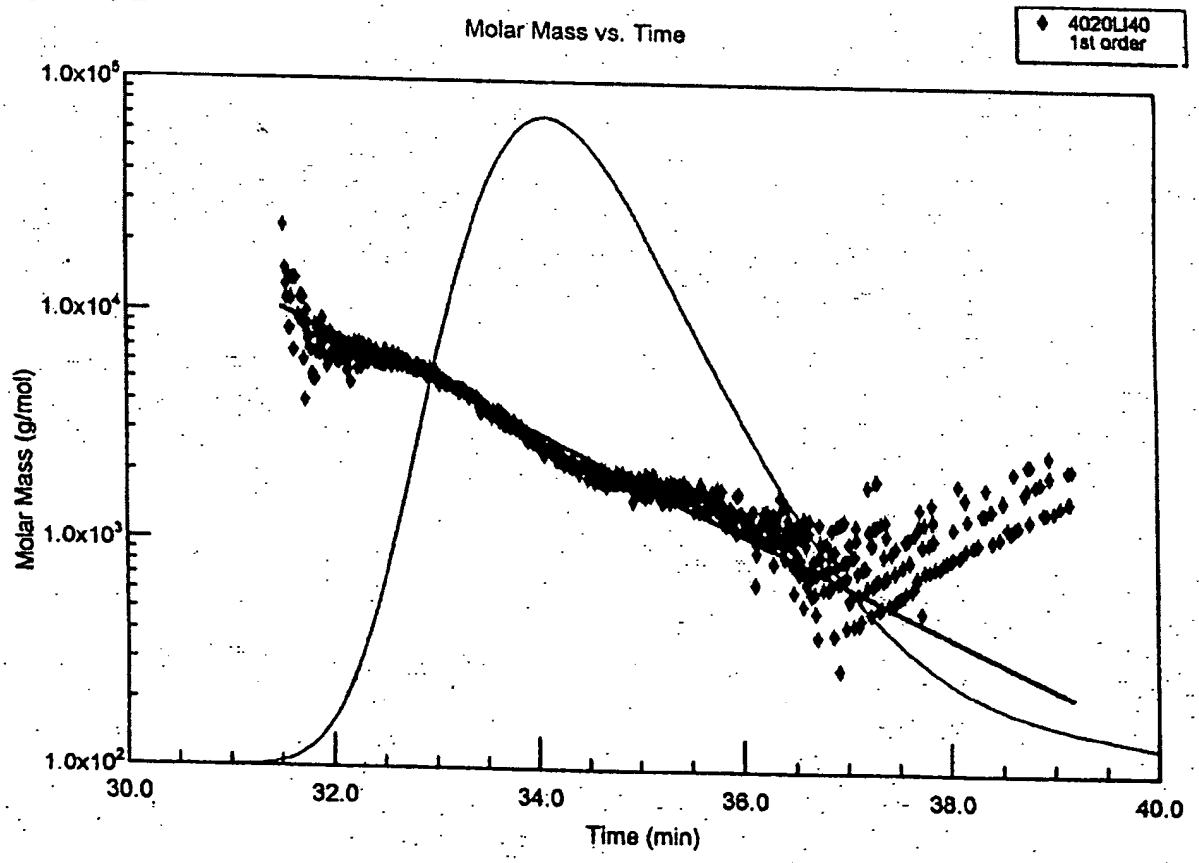


FIG. 15B



Short-branched fructan 4030LI140 @ 2:1 FOS: Sucrose

FIG. 16A

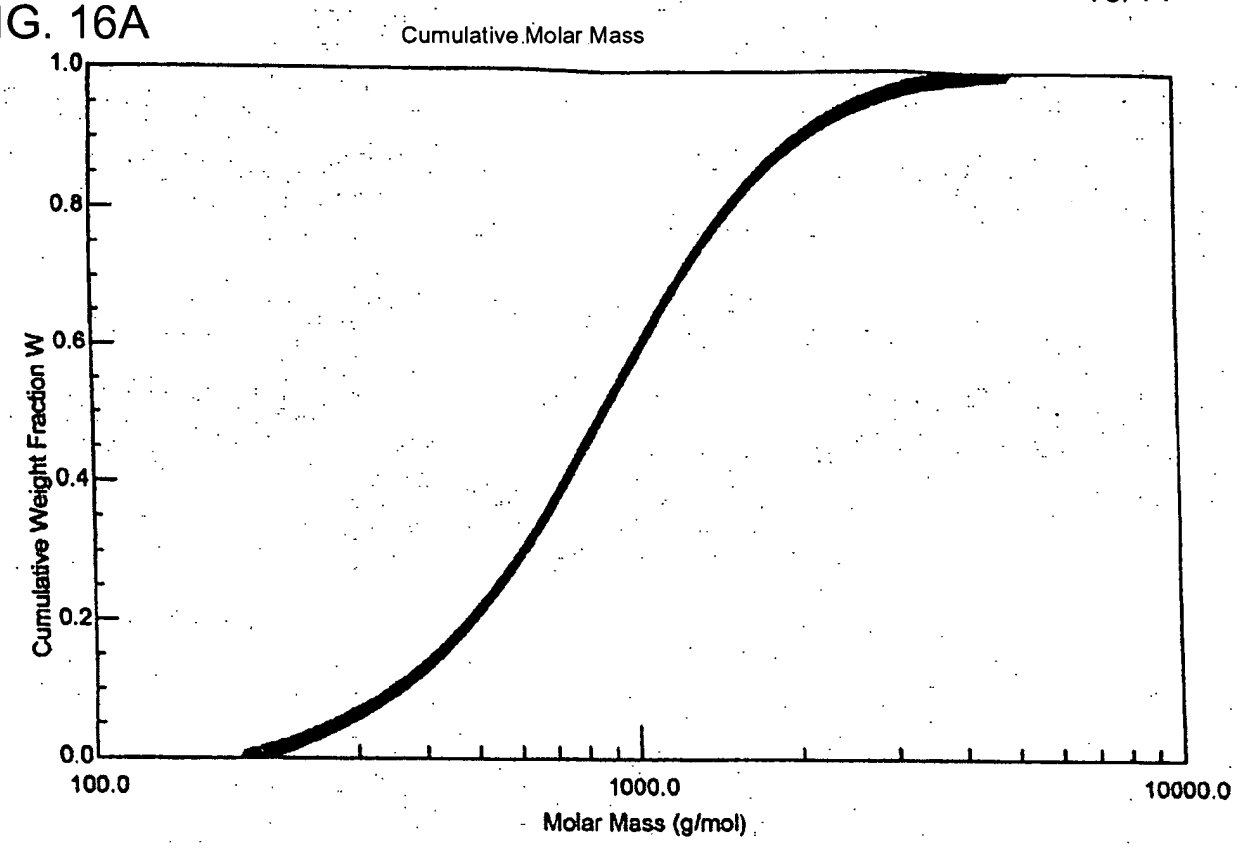
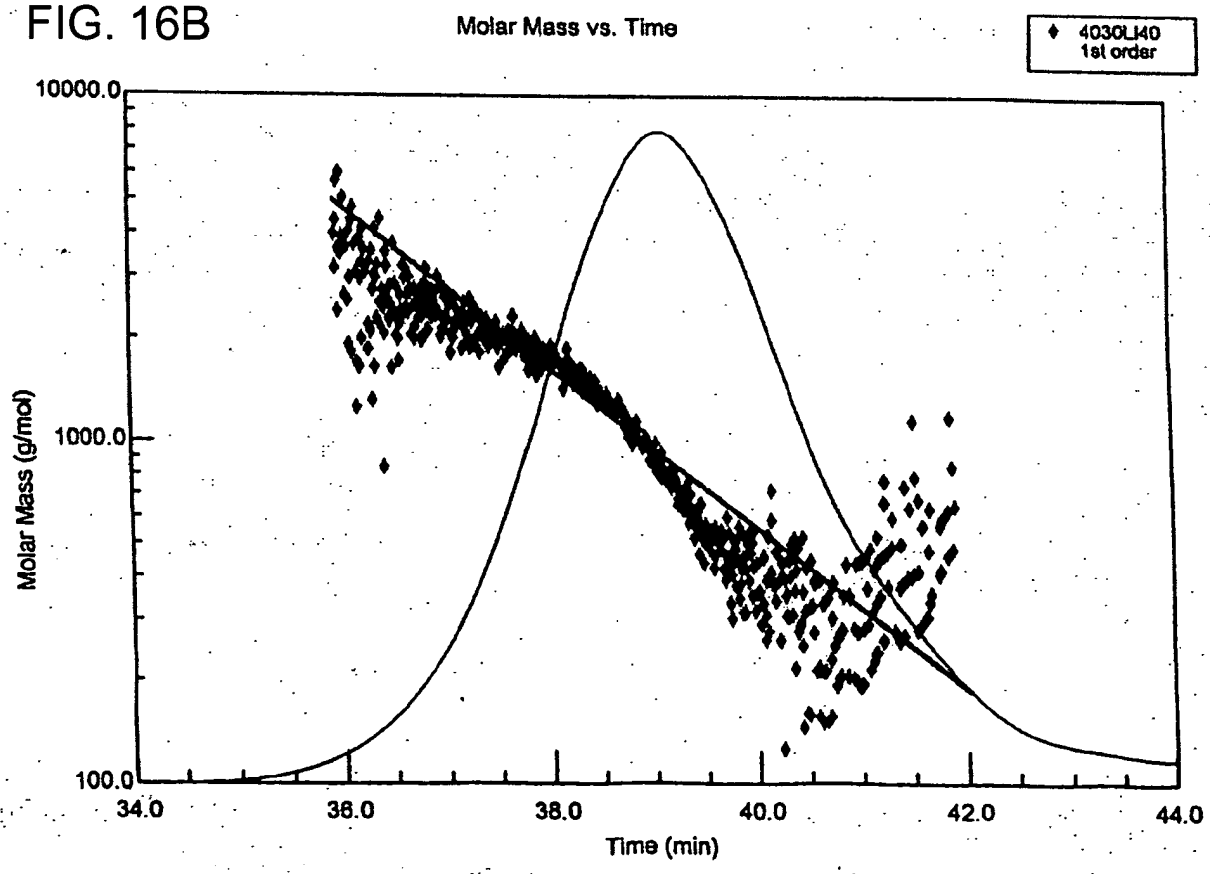
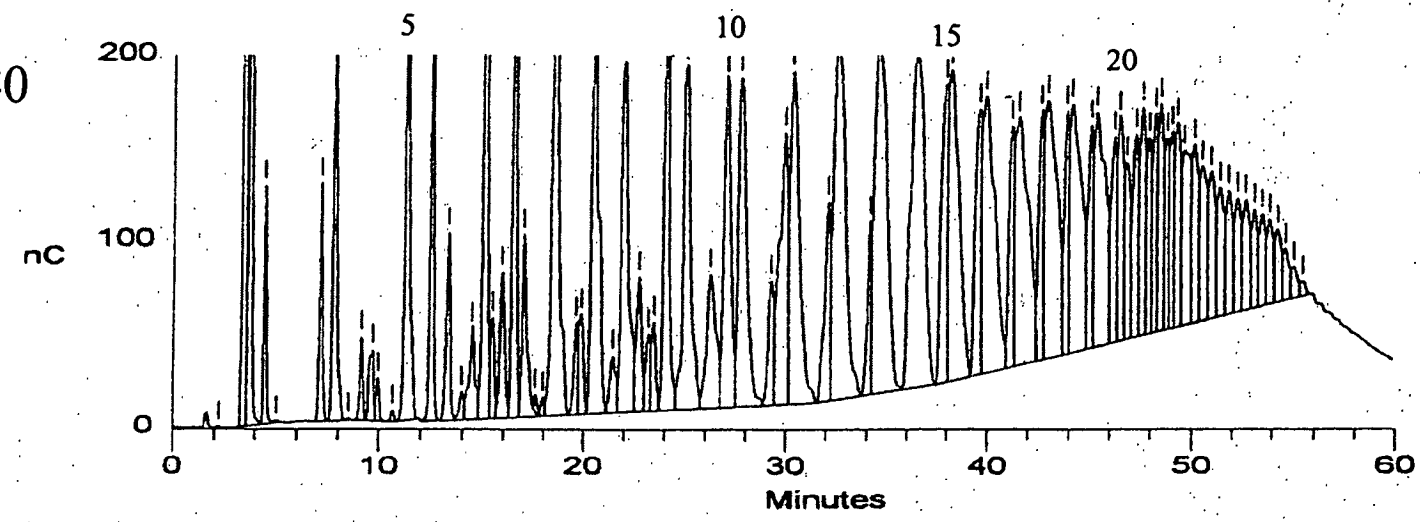


FIG. 16B



Levan 4010LI40
@ 50°C



Levan 4020LI40
@ 30°C

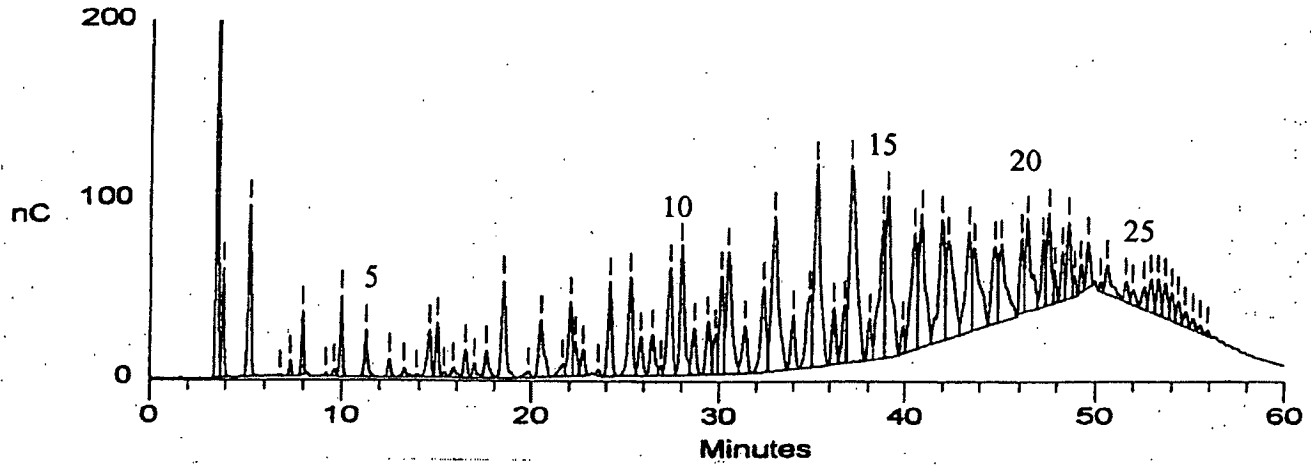
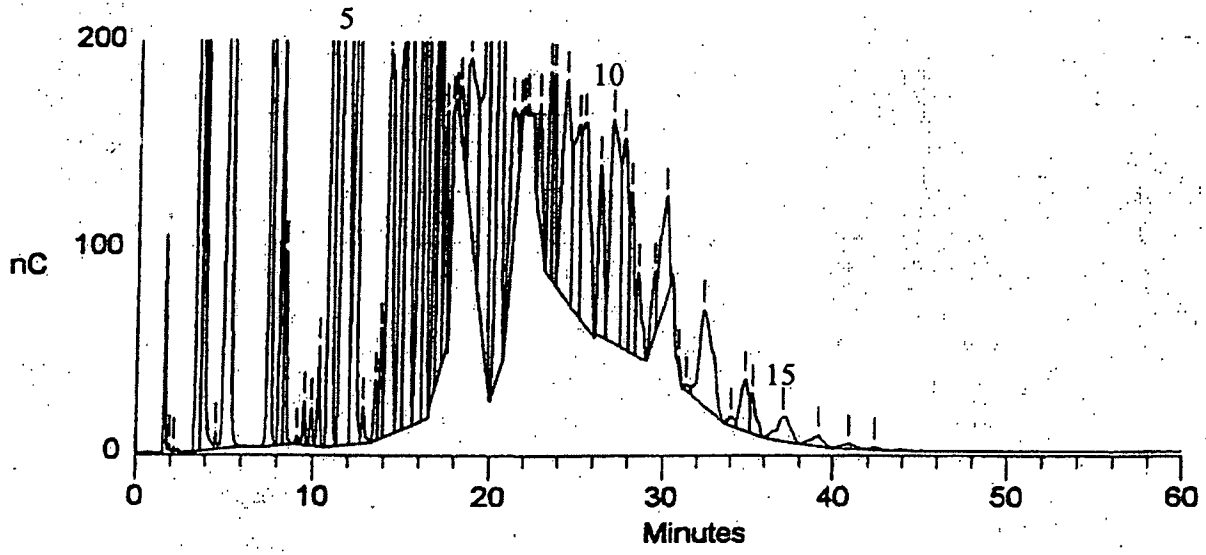


FIG. 17

Short-branched fructan
4030LI40

2:1 FOS:sucrose



Long-branched fructan
5028BI50

1:1 FOS:sucrose

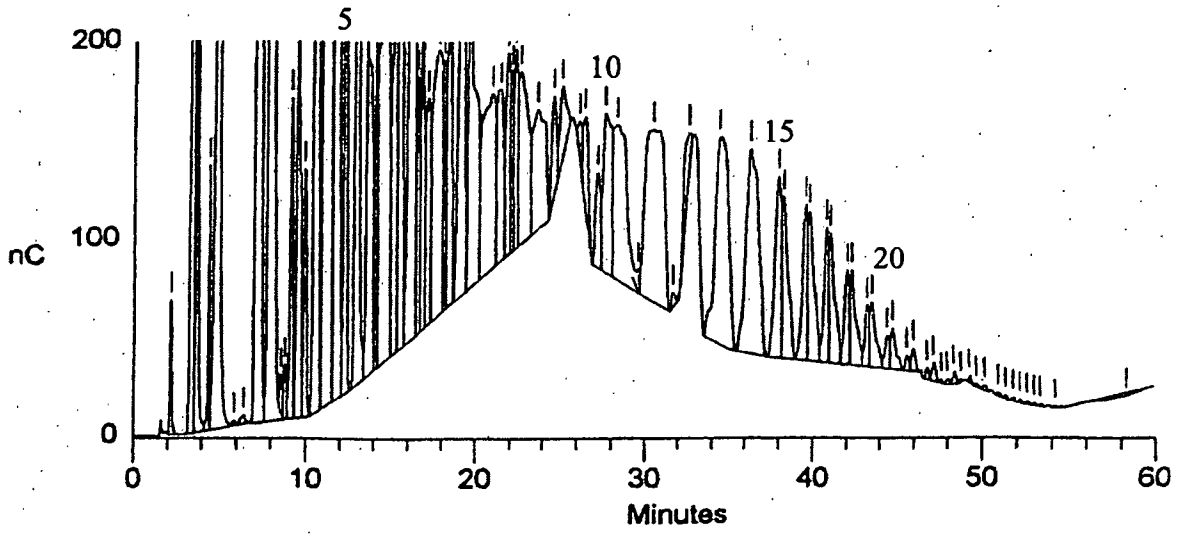


FIG. 18

FOS and branched levan metabolism by Lactobacillus acidophilus ATCC 4357 using MRS media w & w/o 2% sugars at 37 ^{0}C 12% CO2 incubation 11/30/00.

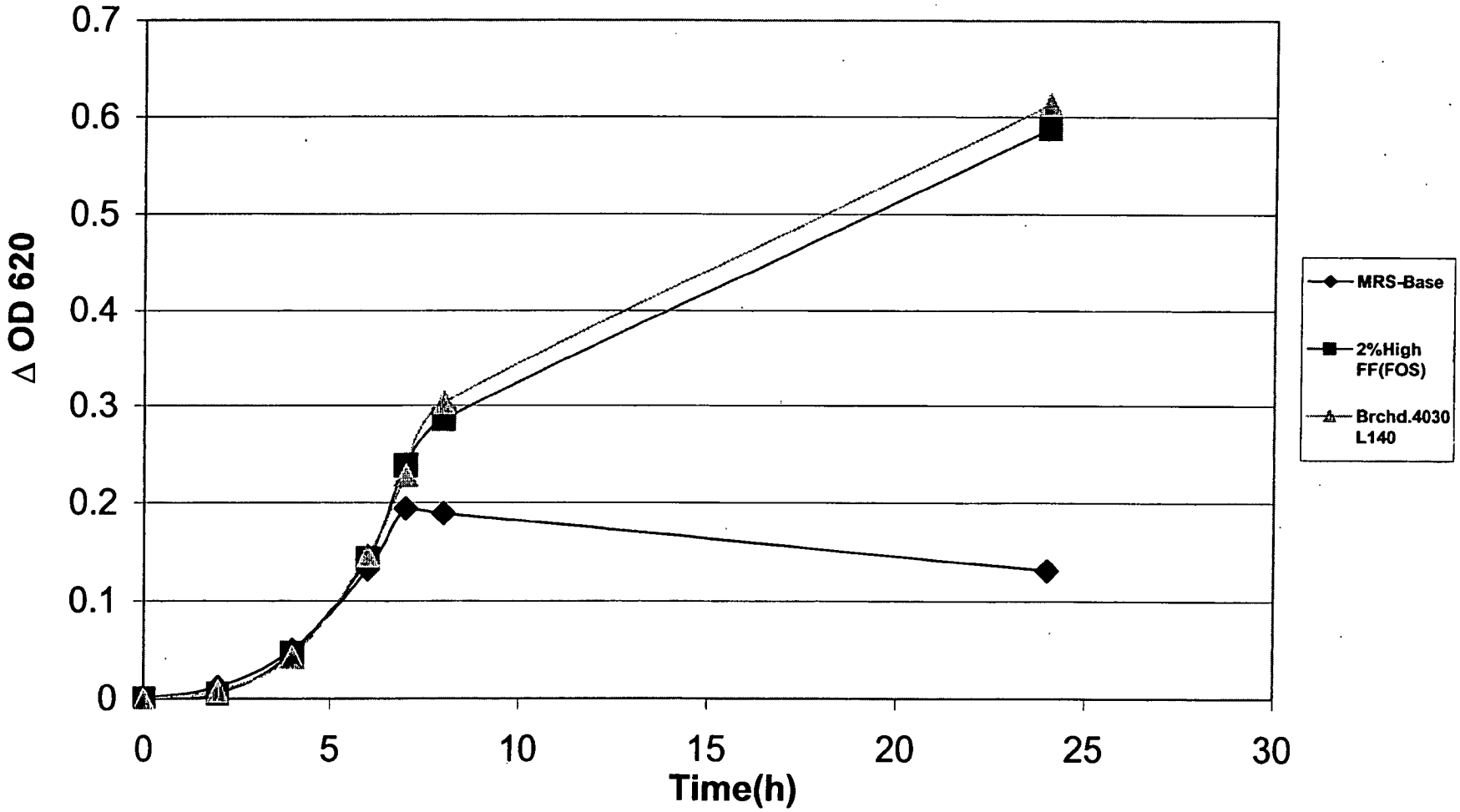


FIG. 19

FOS and branched levan metabolism by Lactobacillus amylovorus ATCC 33620 using MRS media.w & w/o sugars at 37 °C 5% CO2 incubation 12/8/00.

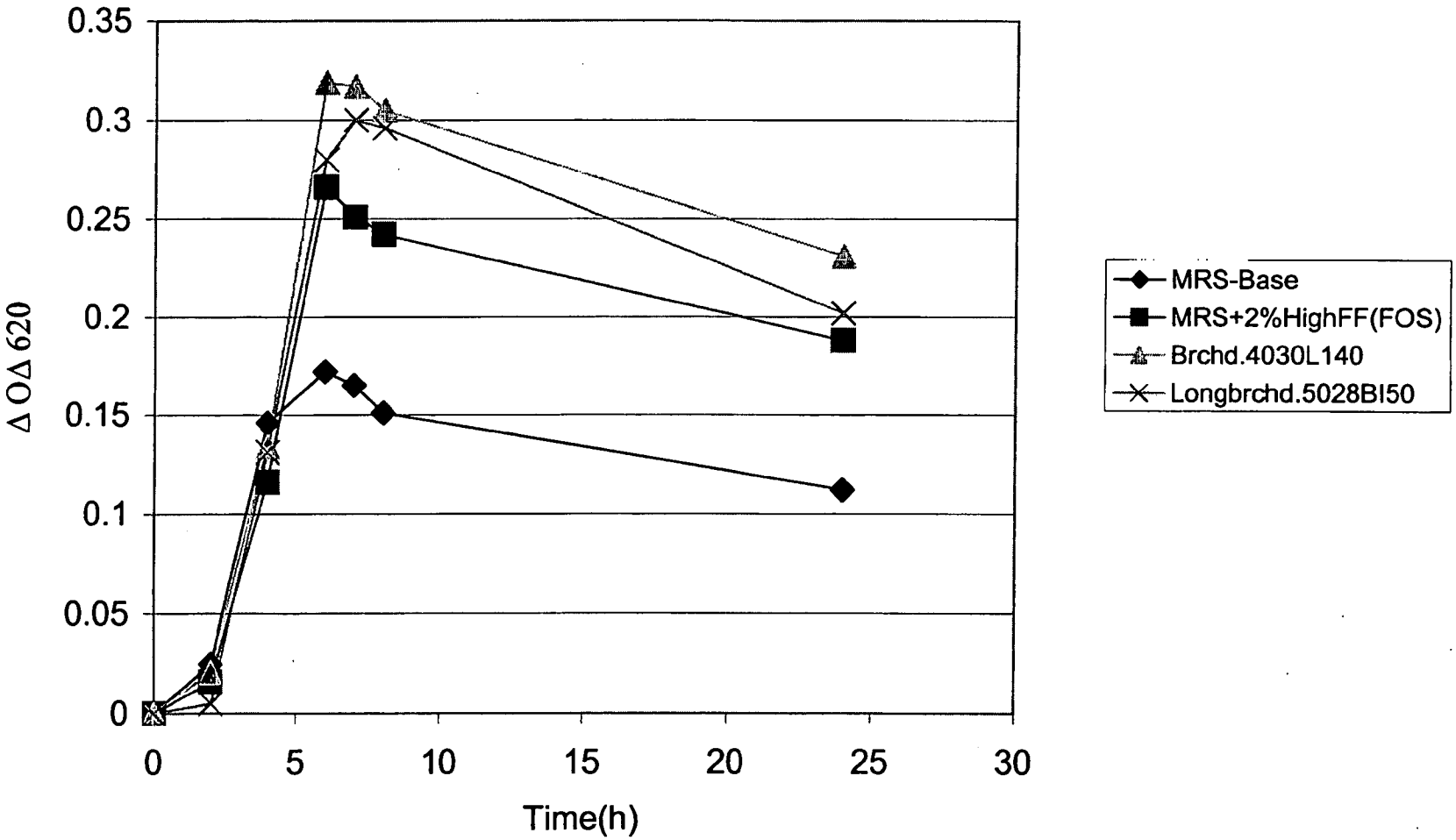


FIG. 20

FOS and branched levan metabolism by Lactobacillus amylovorus ATCC 33198 using MRS media w & w/o 2% sugars at 37^{0}C 5% CO2 incubation.

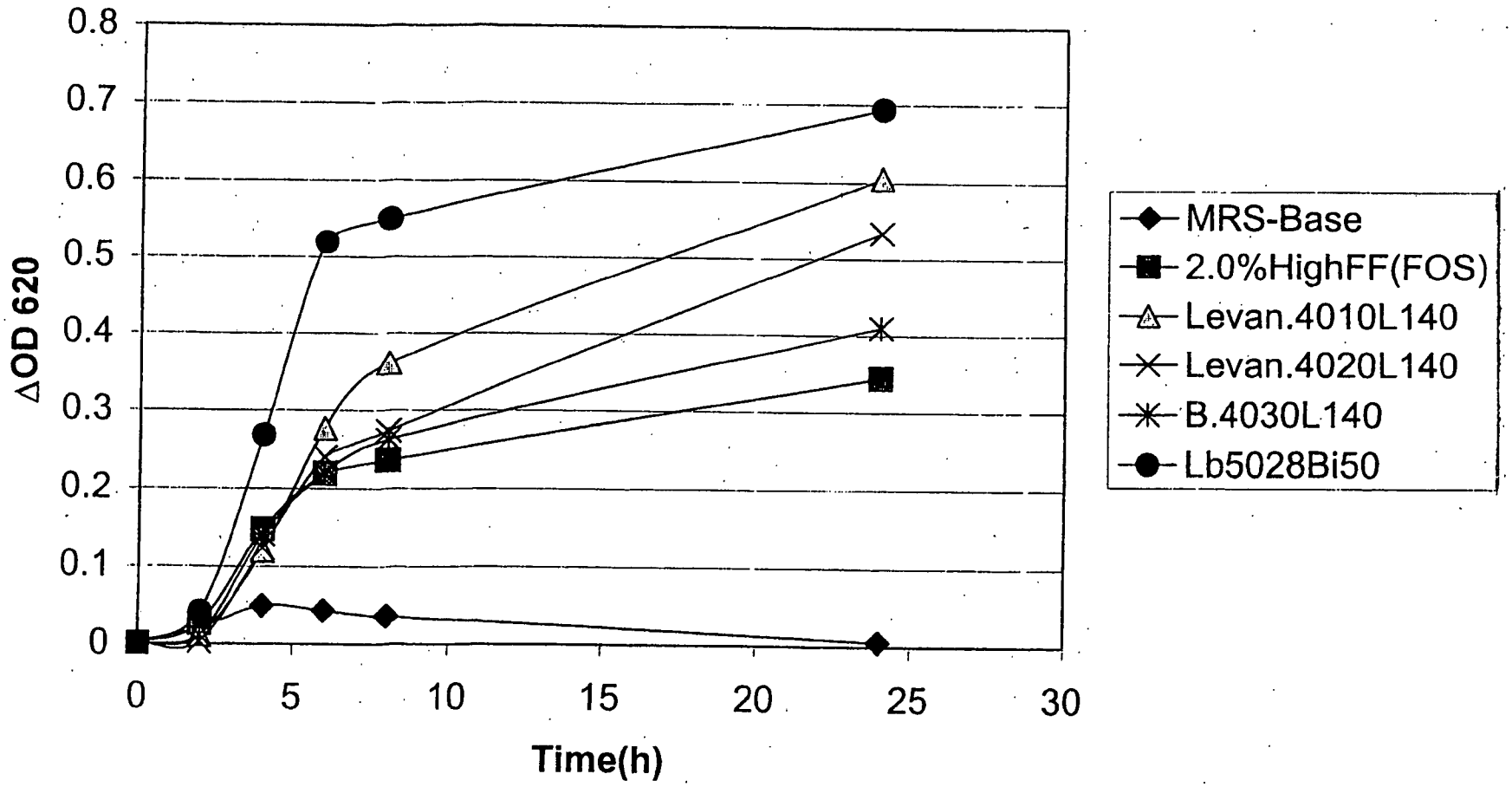


FIG. 21

Fos metabolism by Lactobacillus johnsonii ATCC 33200 using MRS media w & w/o sugars at 37 ^{O}C 5% CO2 incubation 5/23/01.

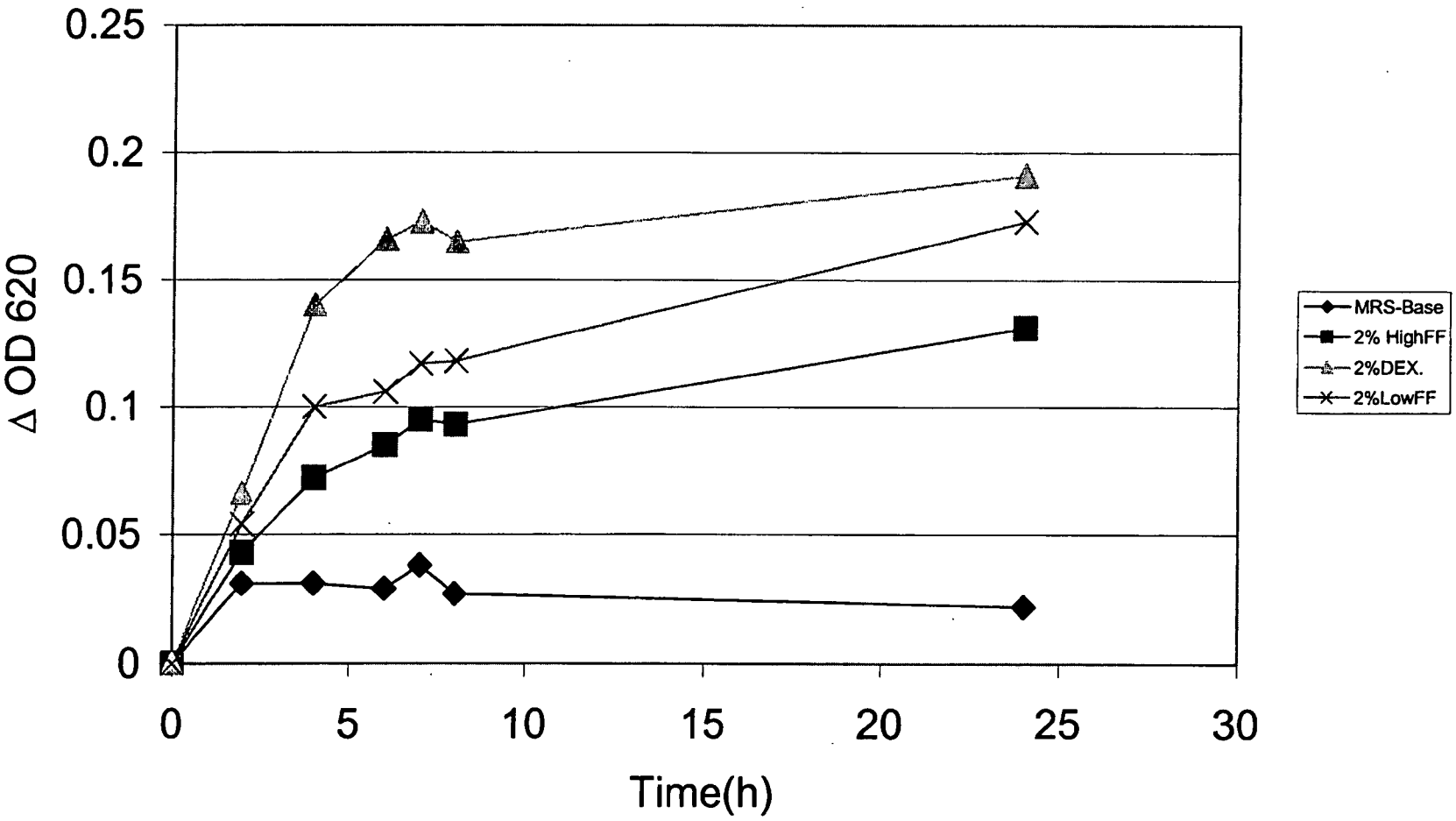


FIG. 22

FOS metabolism by Lactobacillus plantarum ATCC 4008 using MRS media w & w/o sugars at 37 ^{O}C 5% CO2 incubation 5/23/01.

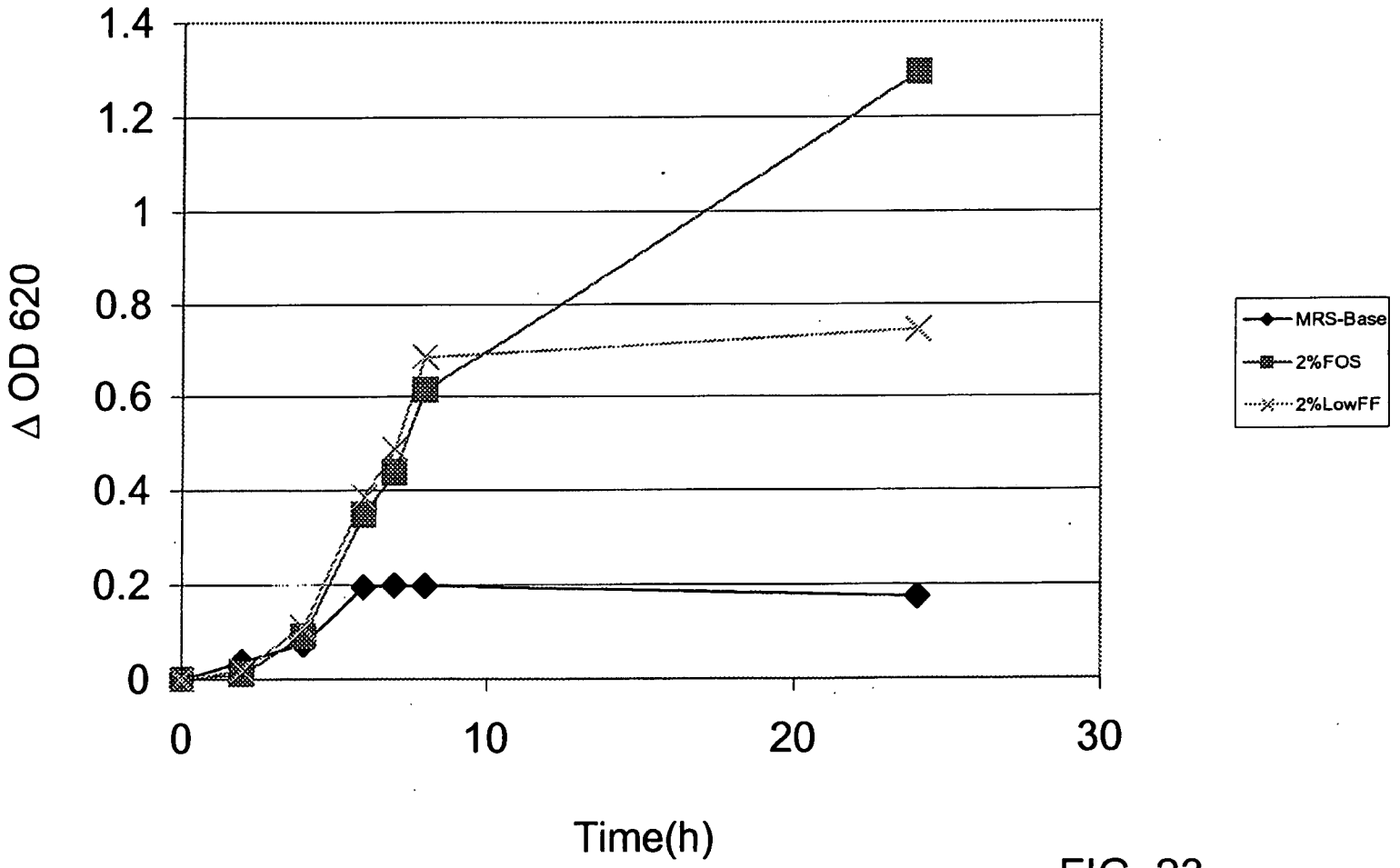


FIG. 23

Sugar metabolism of Escherichia coli ATCC 23502 (O5-K4) using Minimal Broth Davis e & w/o sugars 1/4/01.

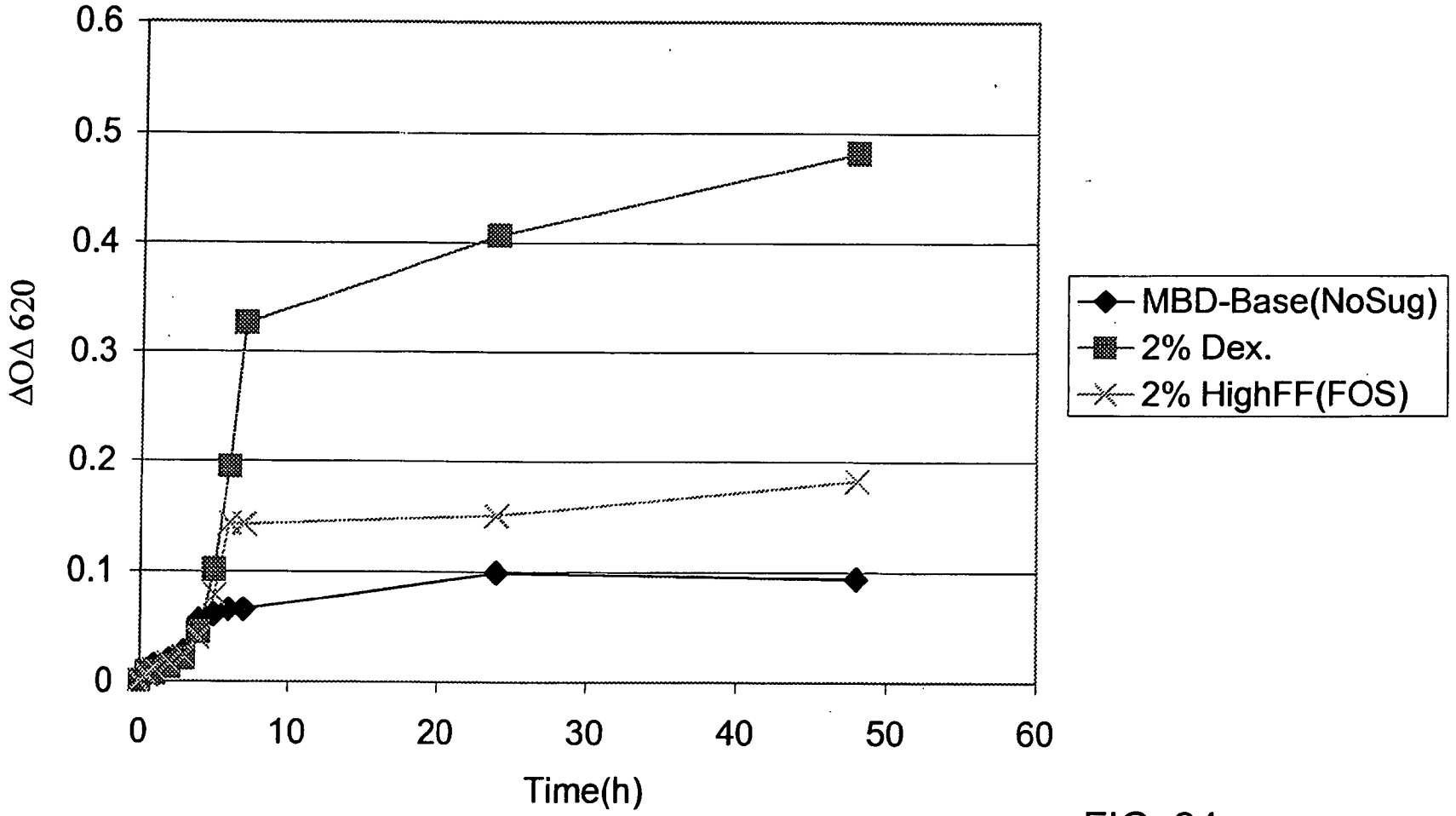


FIG. 24

Sugar metabolism of Escherichia coli U1-41(05-K4) using Minimal Broth Davis w & w/o 2% sugars 1/4/01.

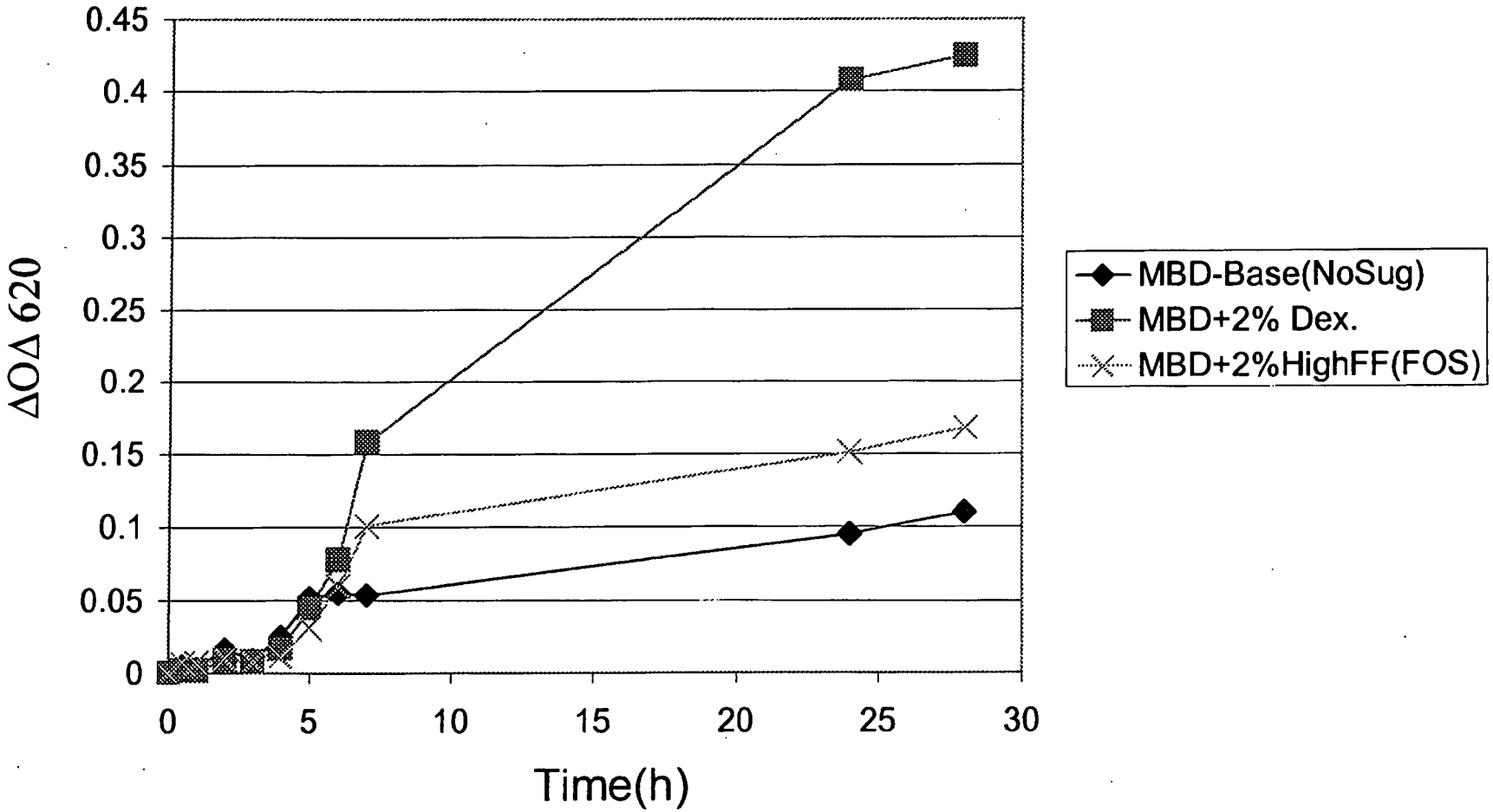


FIG. 25

FOS metabolism by Escherichia coli O157:H7(021901-1) using MBD media w & w/o sugars at 37 °C 5% CO2 incubation 3/1/01.

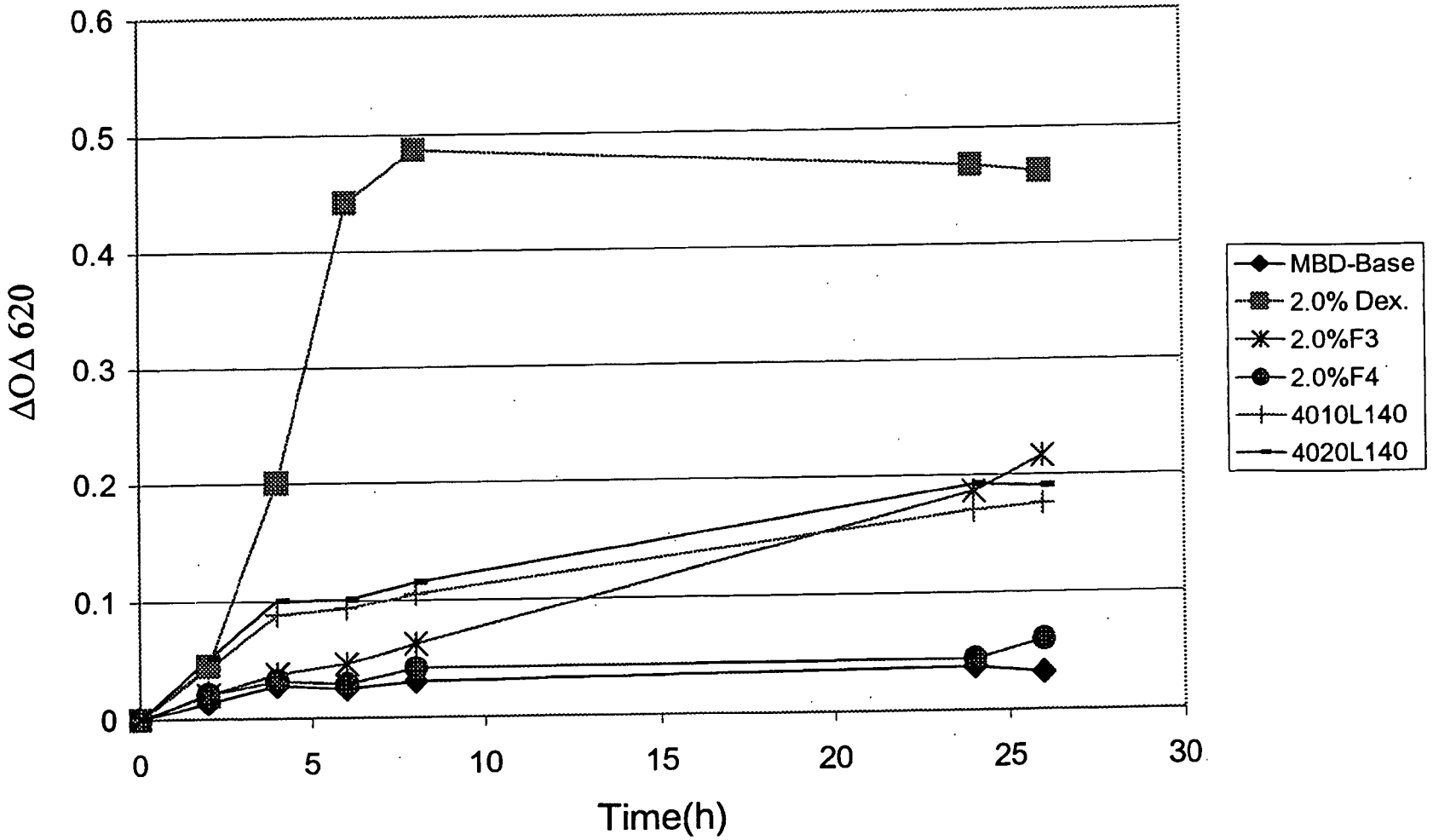


FIG. 26

FOS metabolism by Salmonella species group B(021901-5) using MBD media w & w/o sugars at 37 °C 5% CO2 incubation 3/9/01.

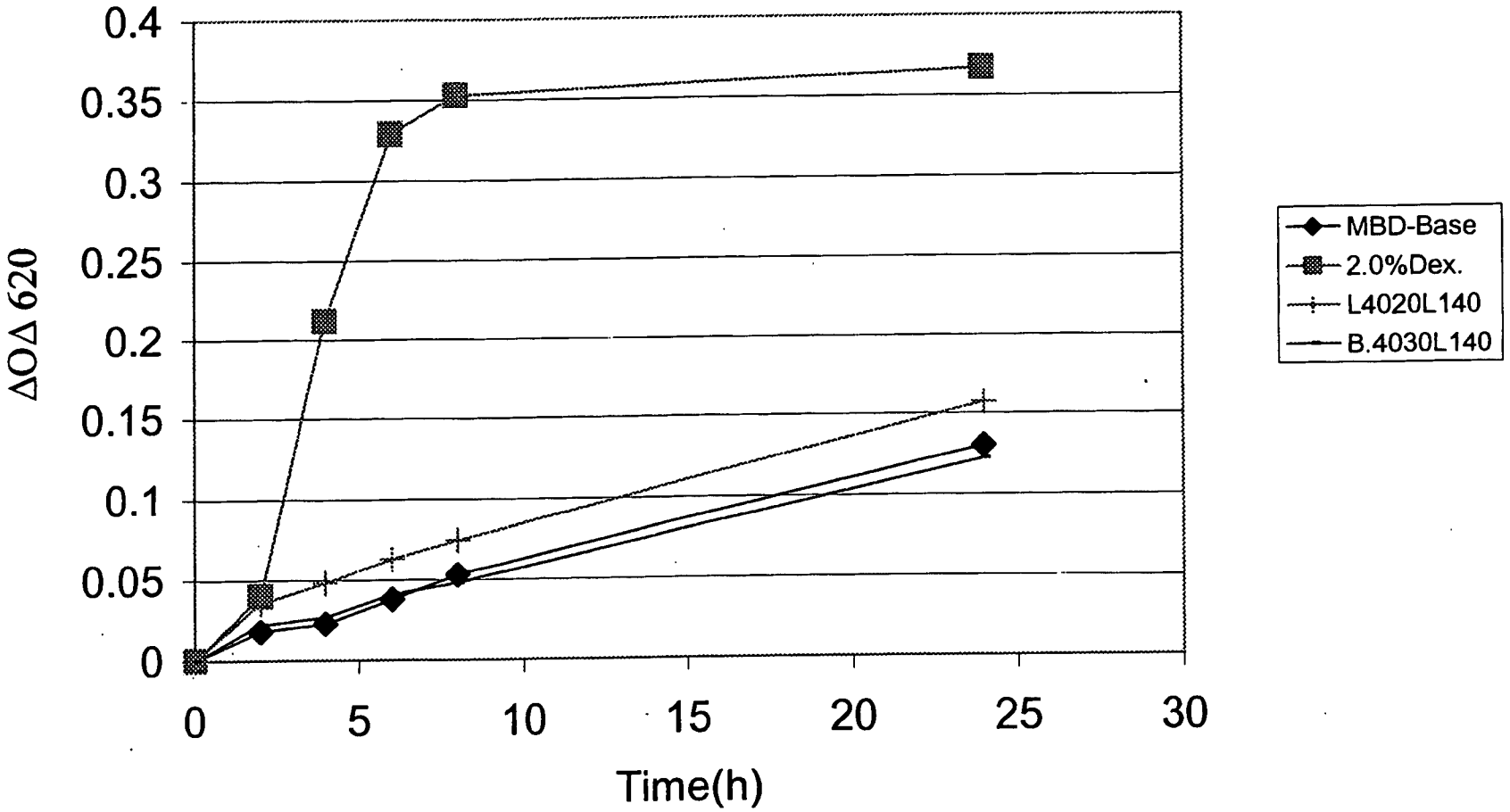


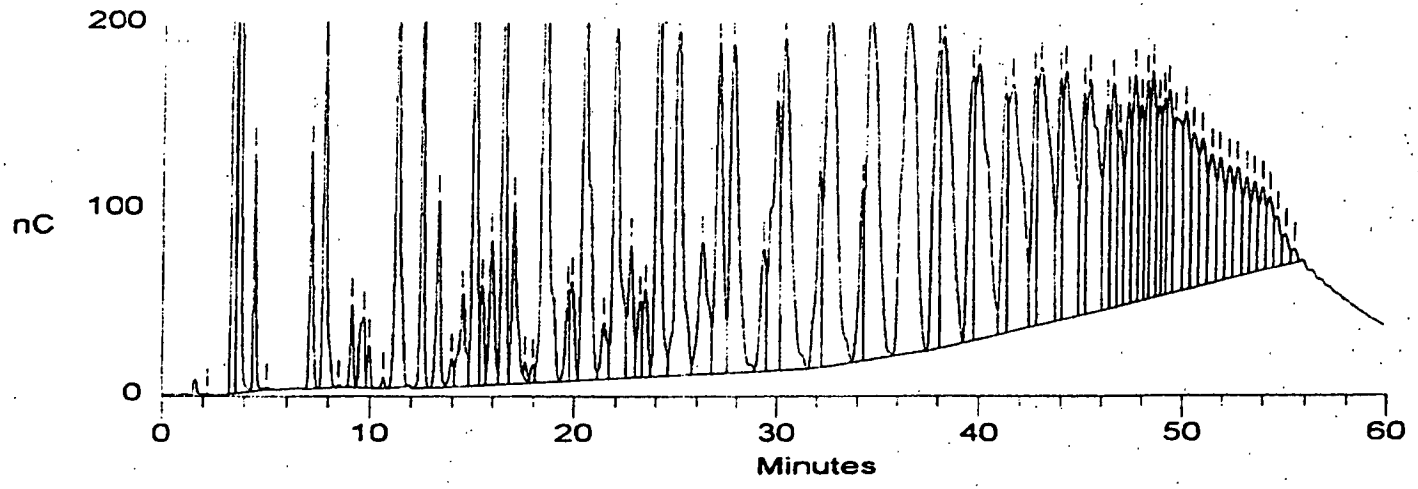
FIG. 27

Cloned Levansucrases

Bacillus subtilis
Gluconacetobacter diazotrophicus
Bacillus stearothermophilus
Pseudomonas aurantiaca
Pseudomonas syringae pv. Glycinea
Rahnella aquatilis
Zymomonas mobilis
Erwinia amylovora
Paenibacillus polymyxa
Acetobacter xylinus
Bacillus amyloliquefaciens

FIG. 28

Levan @ 50°C



Levan @ 30°C

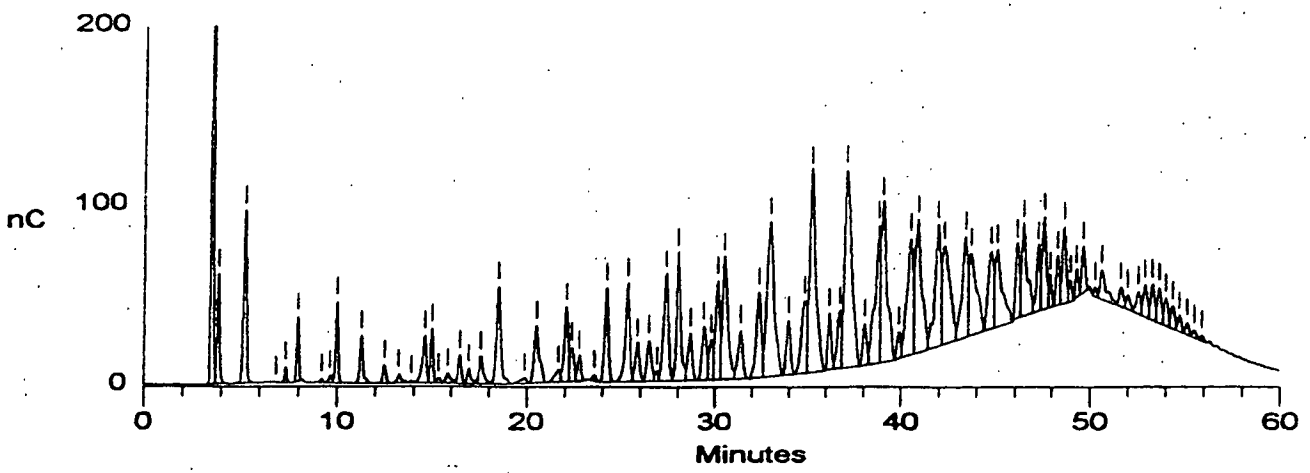


FIG. 29

Levan cGMP batch

Input: 62.88 kg sucrose
150 Units *B. subtilis* levansucrase

Reaction: 81.2 L @ 50°C X 65 hours

	kg levan	kg sucrose	kg glucose	kg fructose
100K filtrate	16.03	4.40	26.40	10.66
post-Amberlite	15.791	4.35	26.00	10.57
G10 retentate	15.99	0.245	0.00	0.00

FIG. 30

MALDI-TOF-MS of 4010LI40

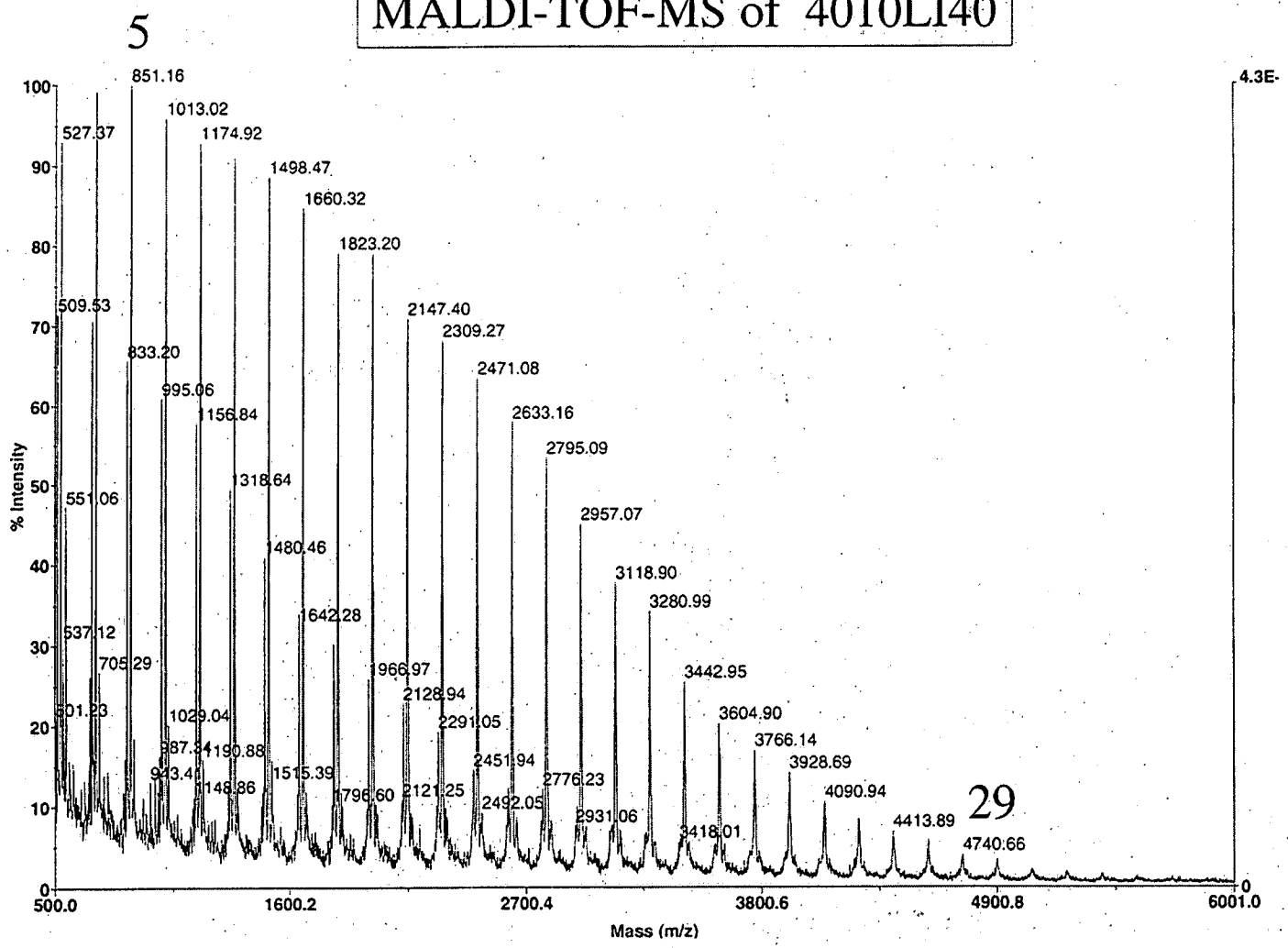


FIG. 31

Size Exclusion / Light Scattering Analysis

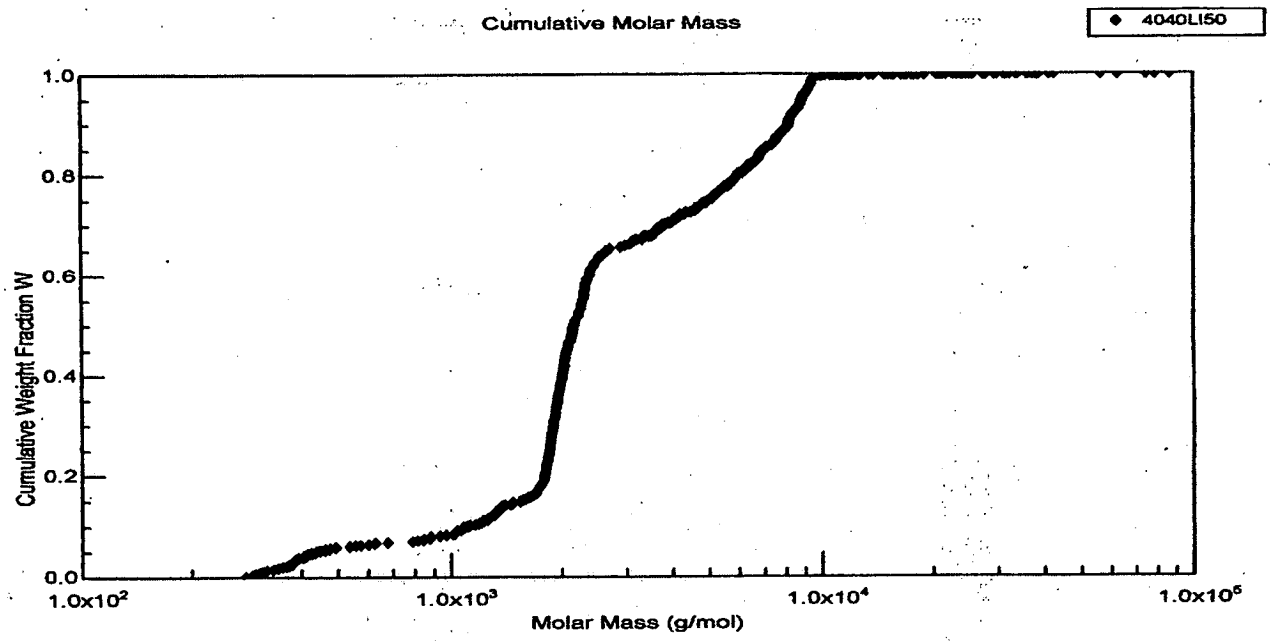


FIG. 32

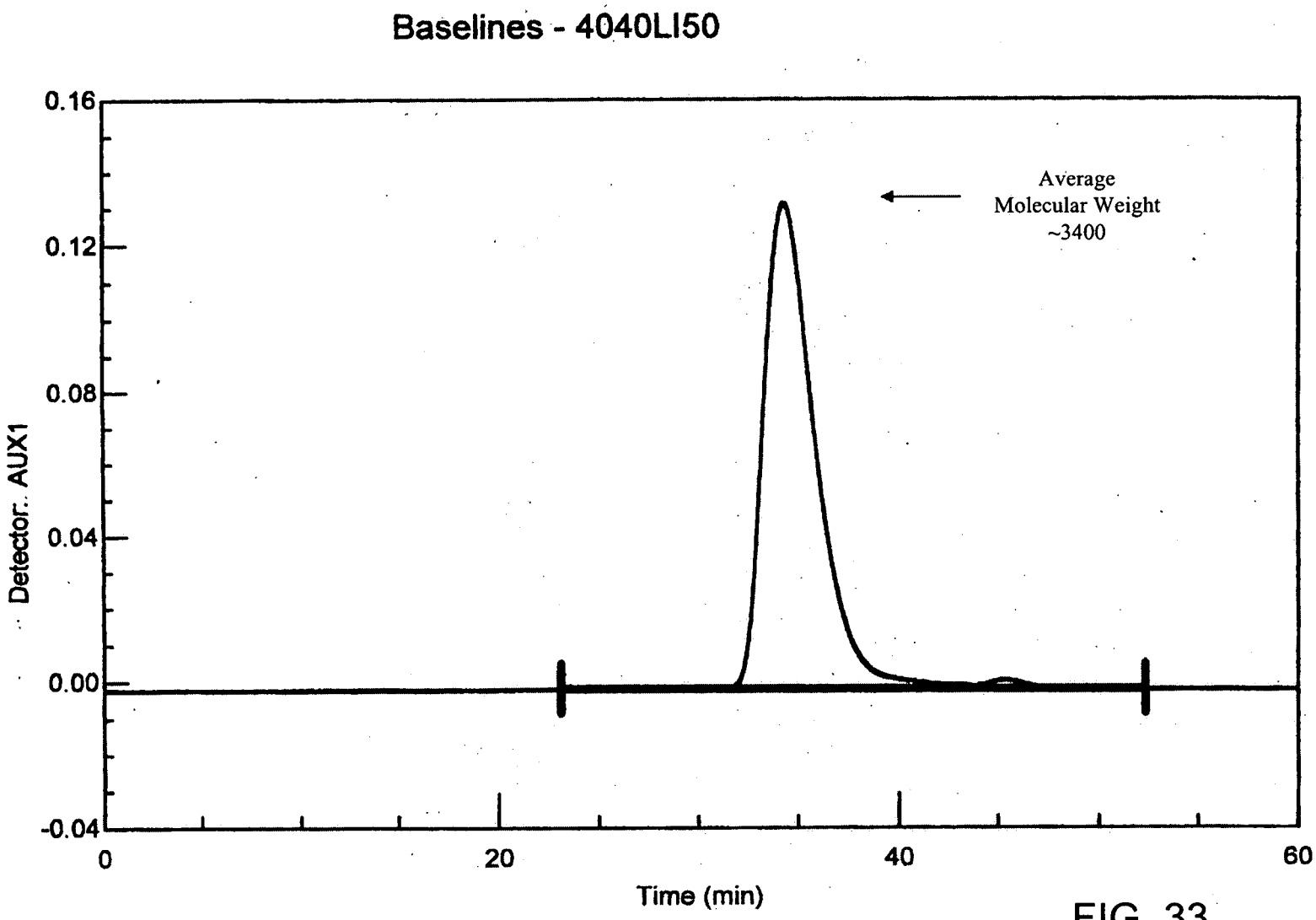

Baselines - 4040LI50
0.16
0.12
0.08
0.04
0.00
-0.04
Detector: AUX1
Average
Molecular Weight
~3400
0
20
40
60
Time (min)

FIG. 33

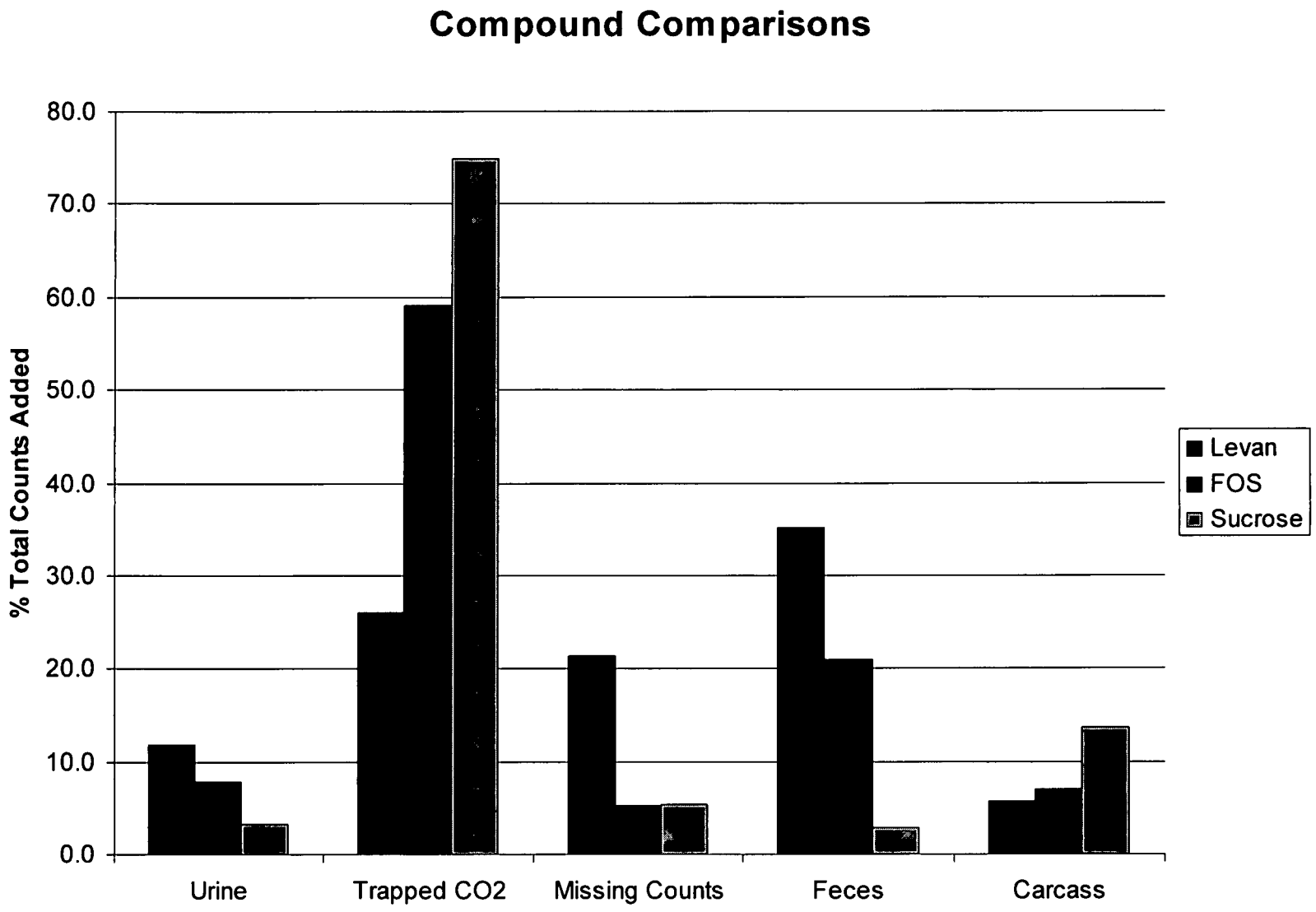

Compound Comparisons
% Total Counts Added
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Urine
Trapped CO2
Missing Counts
Feces
Carcass
Levan
FOS
Sucrose

FIG. 34

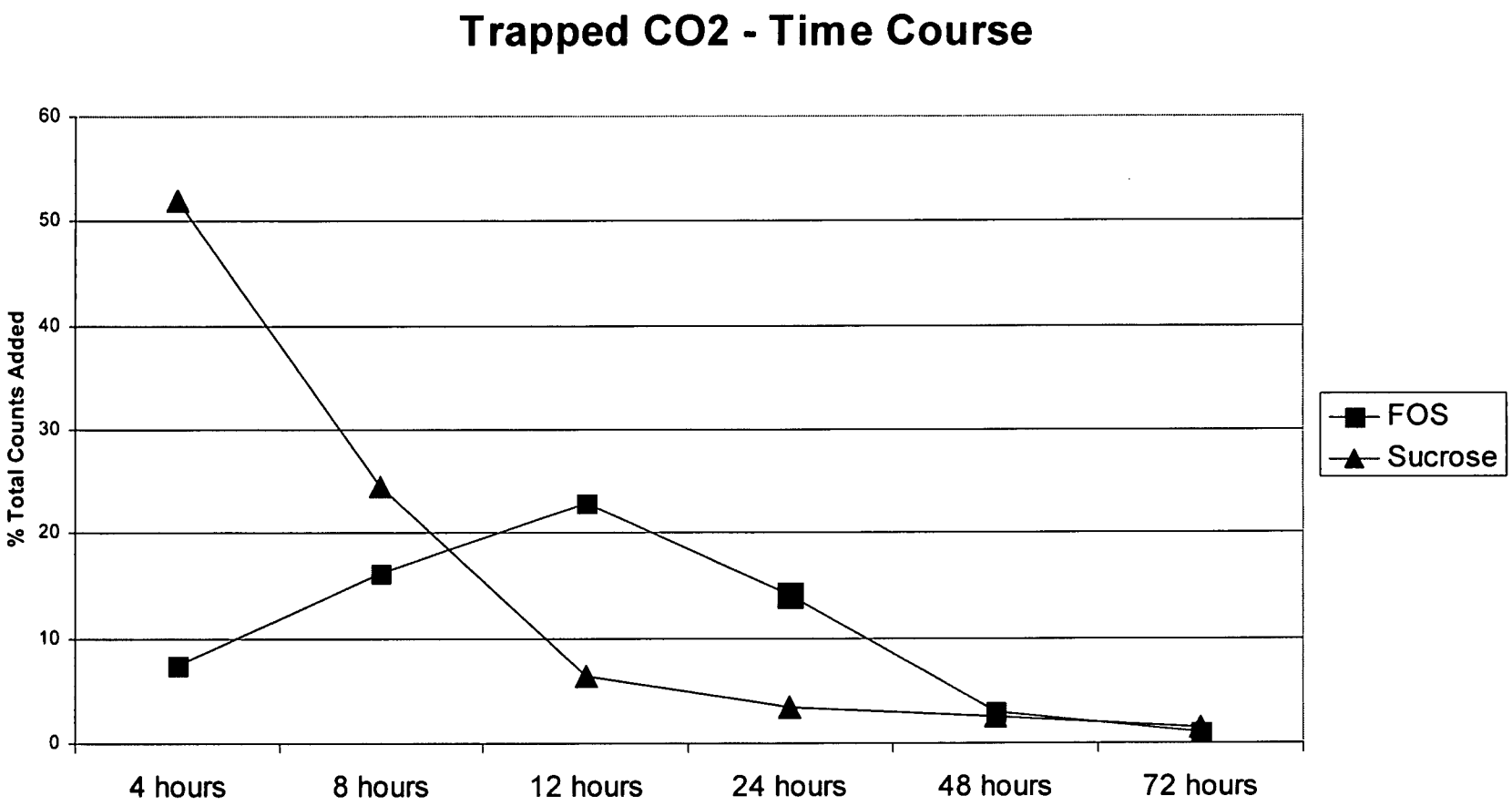

Trapped CO2 - Time Course
% Total Counts Added
60
50
40
30
20
10
0
4 hours
8 hours
12 hours
24 hours
48 hours
72 hours
FOS
Sucrose

FIG. 35

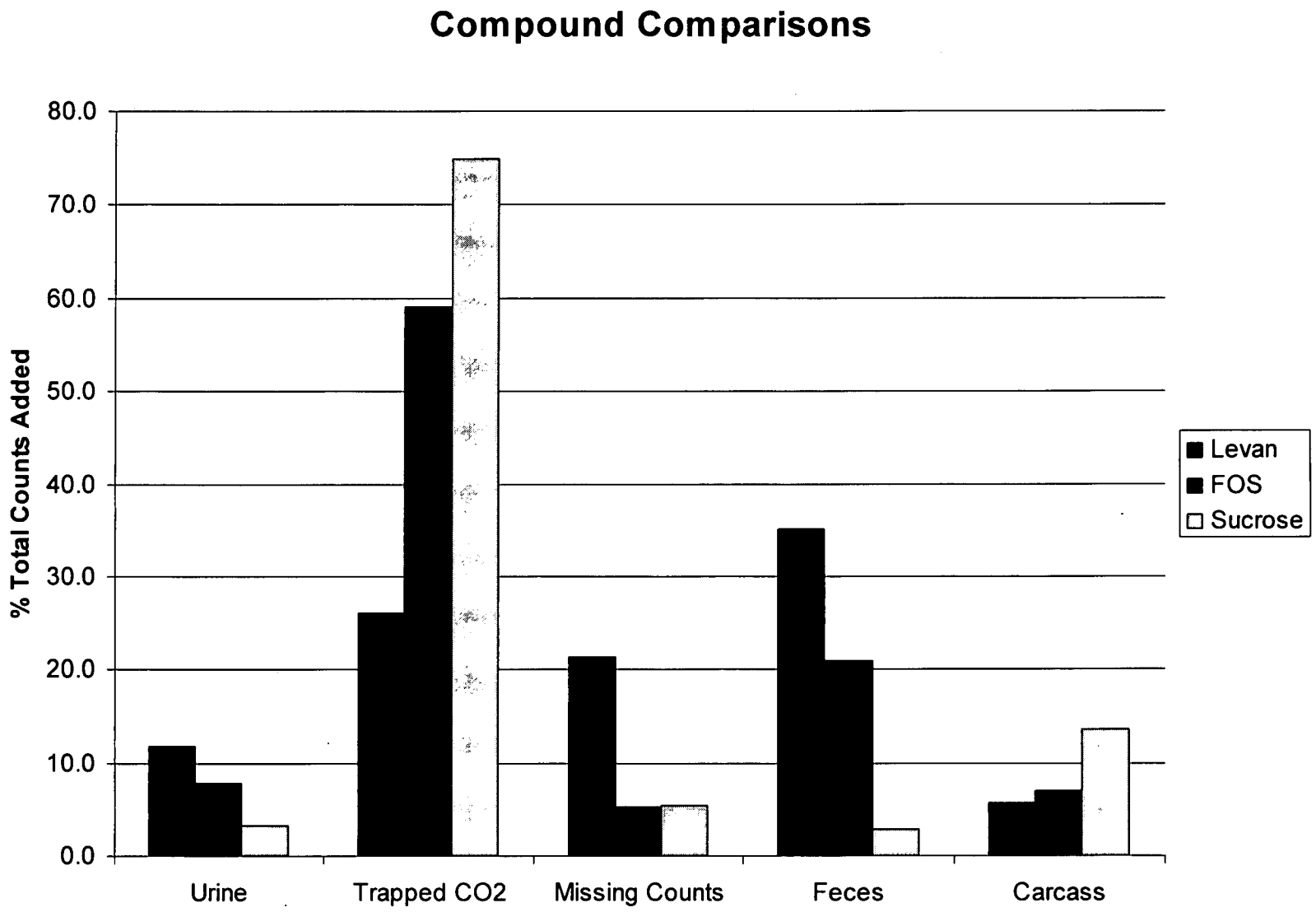

Compound Comparisons
% Total Counts Added
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Urine
Trapped CO2
Missing Counts
Feces
Carcass
Levan
FOS
Sucrose

FIG. 36

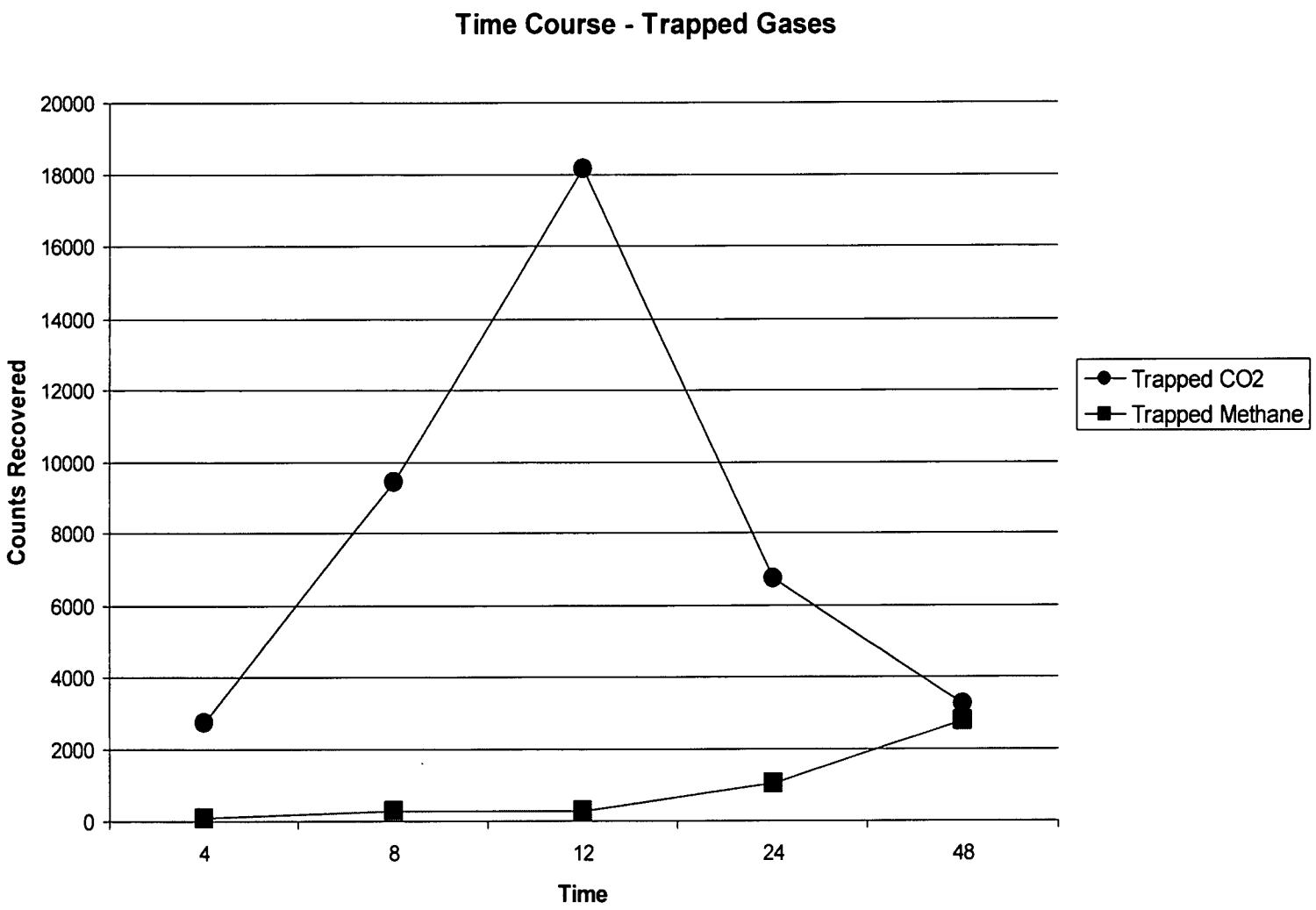

Time Course - Trapped Gases
Counts Recovered
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
4
8
12
24
48
Time
Trapped CO2
Trapped Methane

FIG. 37

Branched fructan GMP batch

Input: 17.8 kg FOS, 9.3 kg sucrose (~2:1 ratio)
15 Units *B. subtilis* levansucrase

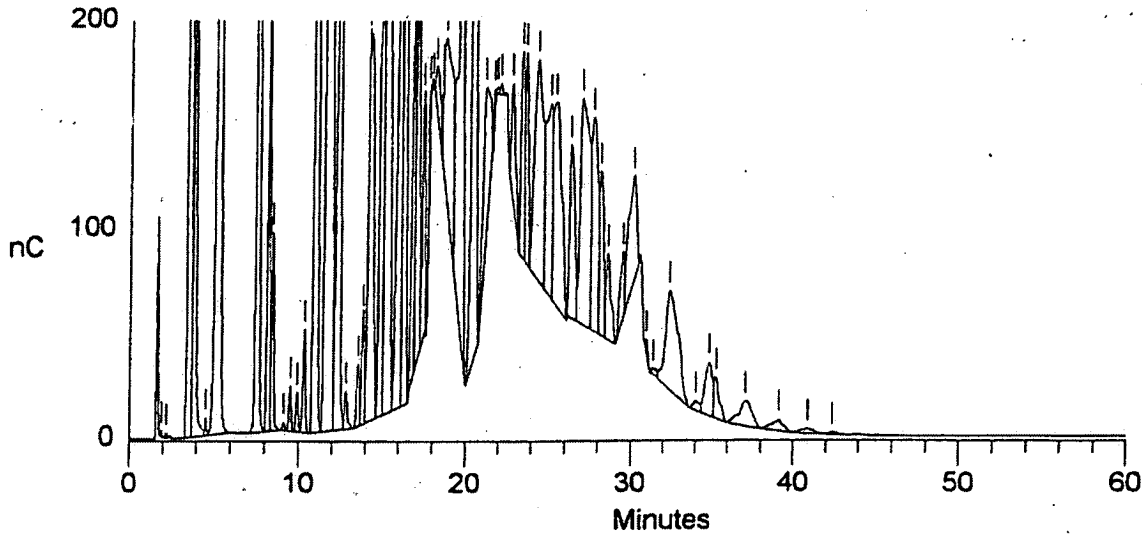
Reaction: 36.1 L @ 50°C X 24 hours

	kg br. fructan	kg sucrose	kg glucose
100K filtrate	20.84	0.43	4.74
Post-Amberlite	19.47	0.40	4.39
G10 retentate	18.39	0.13	0.21

FIG. 38

Branched fructan @ 50°C

2:1 FOS:sucrose



Branched fructan @ 35°C

1:1 FOS:sucrose

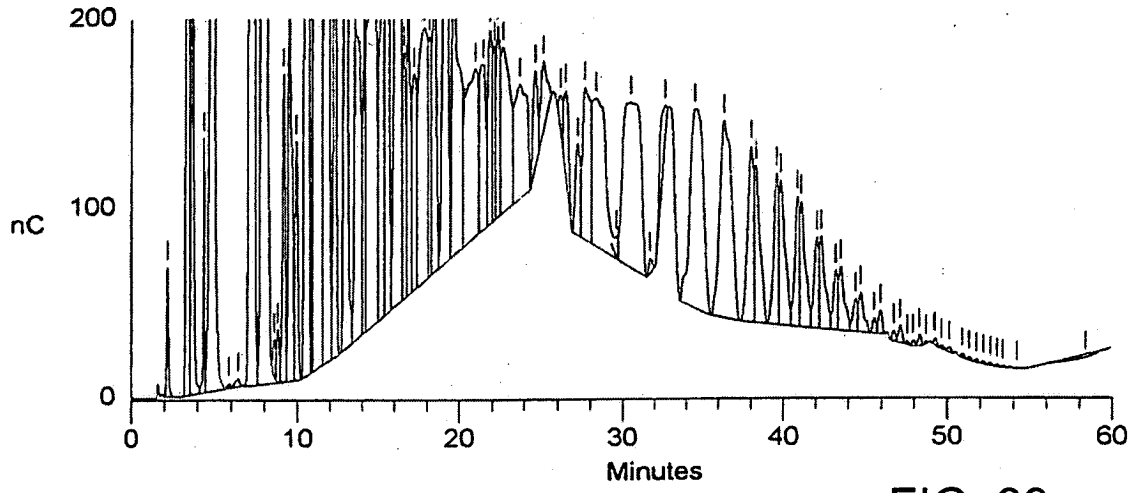


FIG. 39

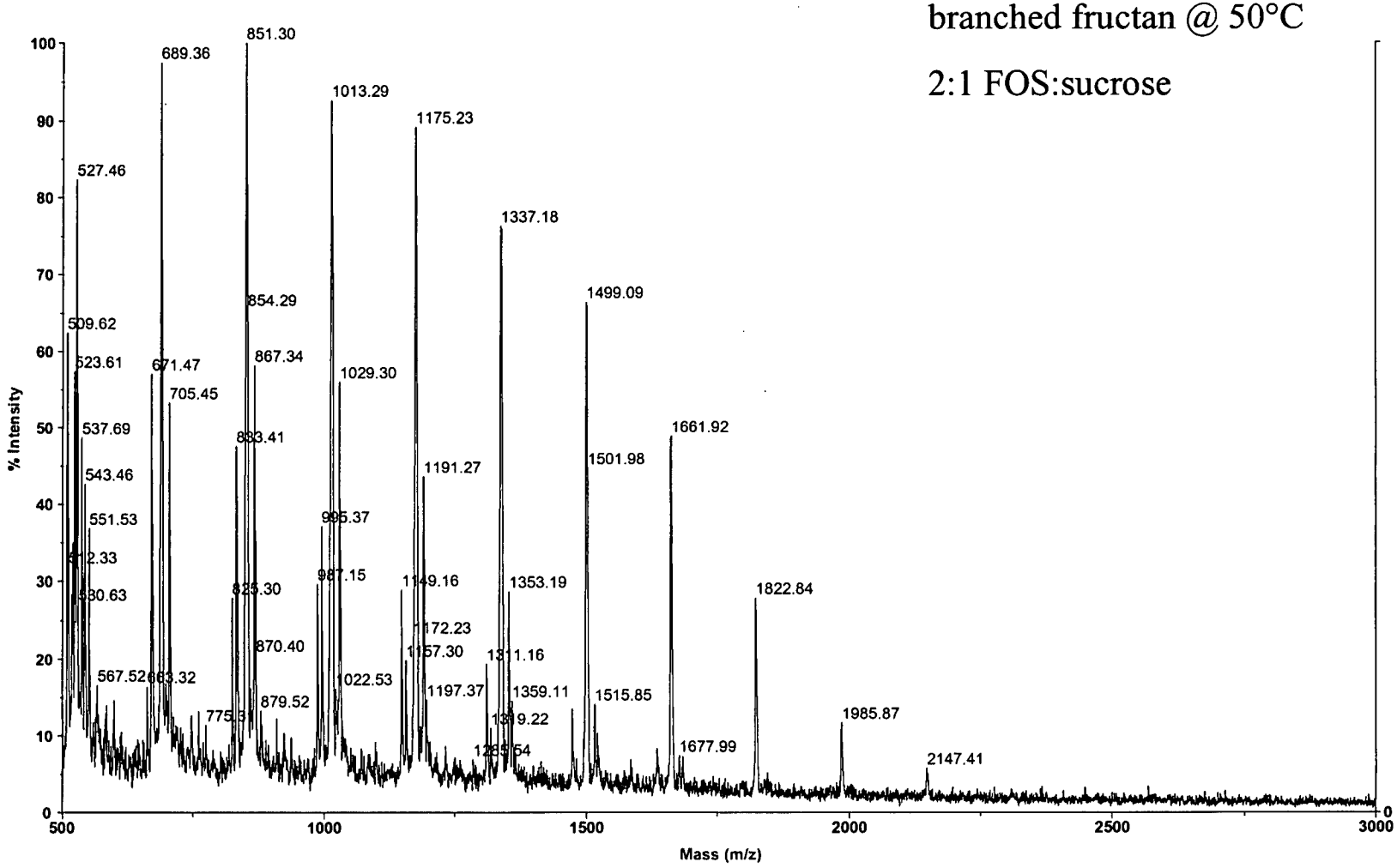

branched fructan @ 50°C
2:1 FOS:sucrose
% Intensity
Mass (m/z)
851.30
689.36
1013.29
1175.23
527.46
1337.18
1499.09
854.29
509.62
867.34
523.61
671.47
1029.30
705.45
1661.92
537.69
1191.27
543.46
1501.98
995.37
551.53
1353.19
1822.84
1985.87
2147.41
1677.99
1515.85
1359.11
1197.37
1022.53
879.52
567.52
775.3

FIG. 40

Branched Fructan Alternate Synthesis

Branched fructan can be synthesized from sucrose with two enzymes simultaneously.

Input: 4.6 kg sucrose
A. niger fructosyltransferase
B. subtilis levansucrase
(Total of 35 Units of both enzymes.)

Reaction: 6L @ 50°C X 16 hours

Reaction #1: 4:1 fructosyltransferase:levansucrase

Reaction #2: 1:1 fructosyltransferase:levansucrase

Reaction #3: 1:4 fructosyltransferase:levansucrase

FIG. 41

Branched fructan reaction #1

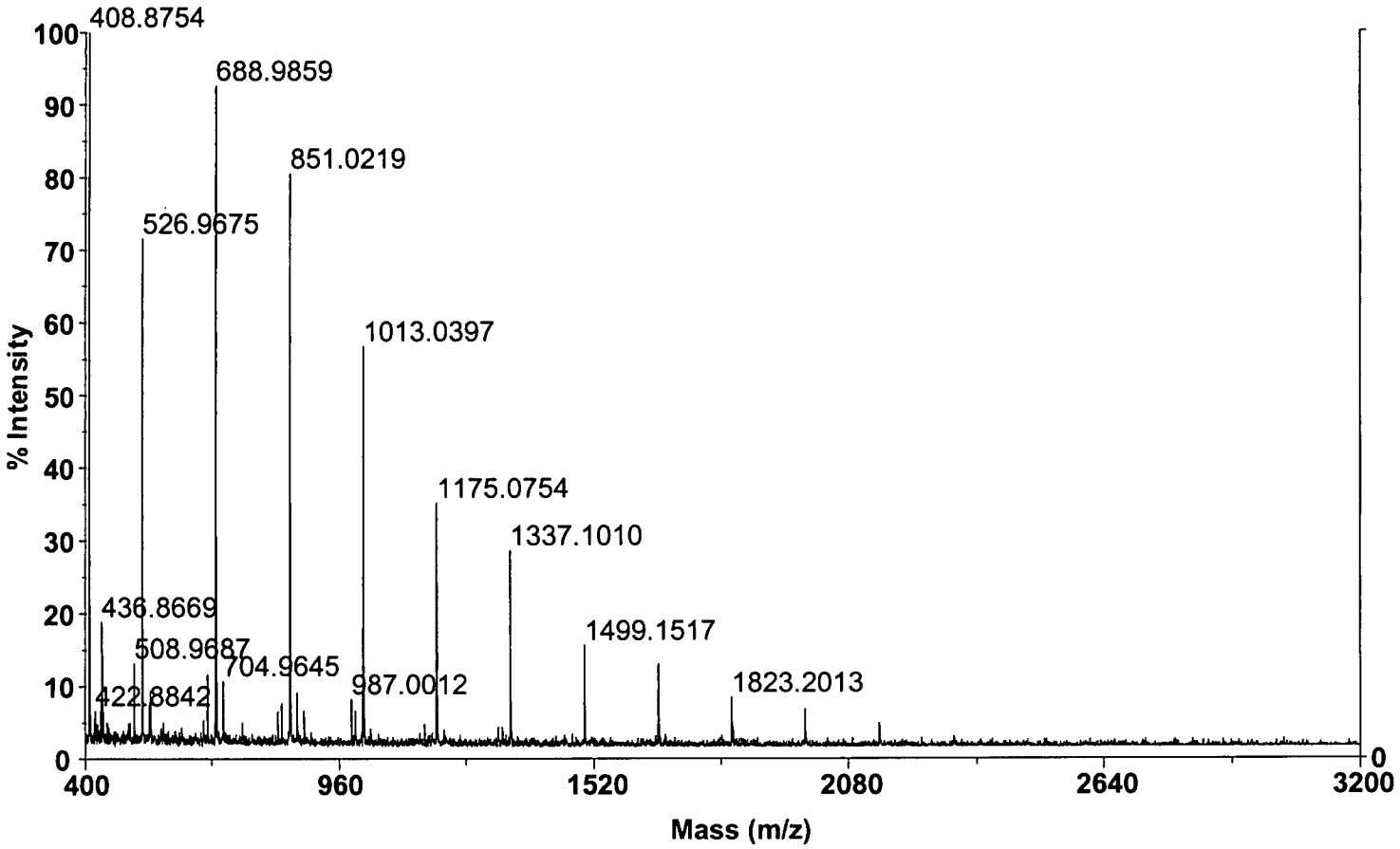


FIG. 42

Branched fructan reaction #2

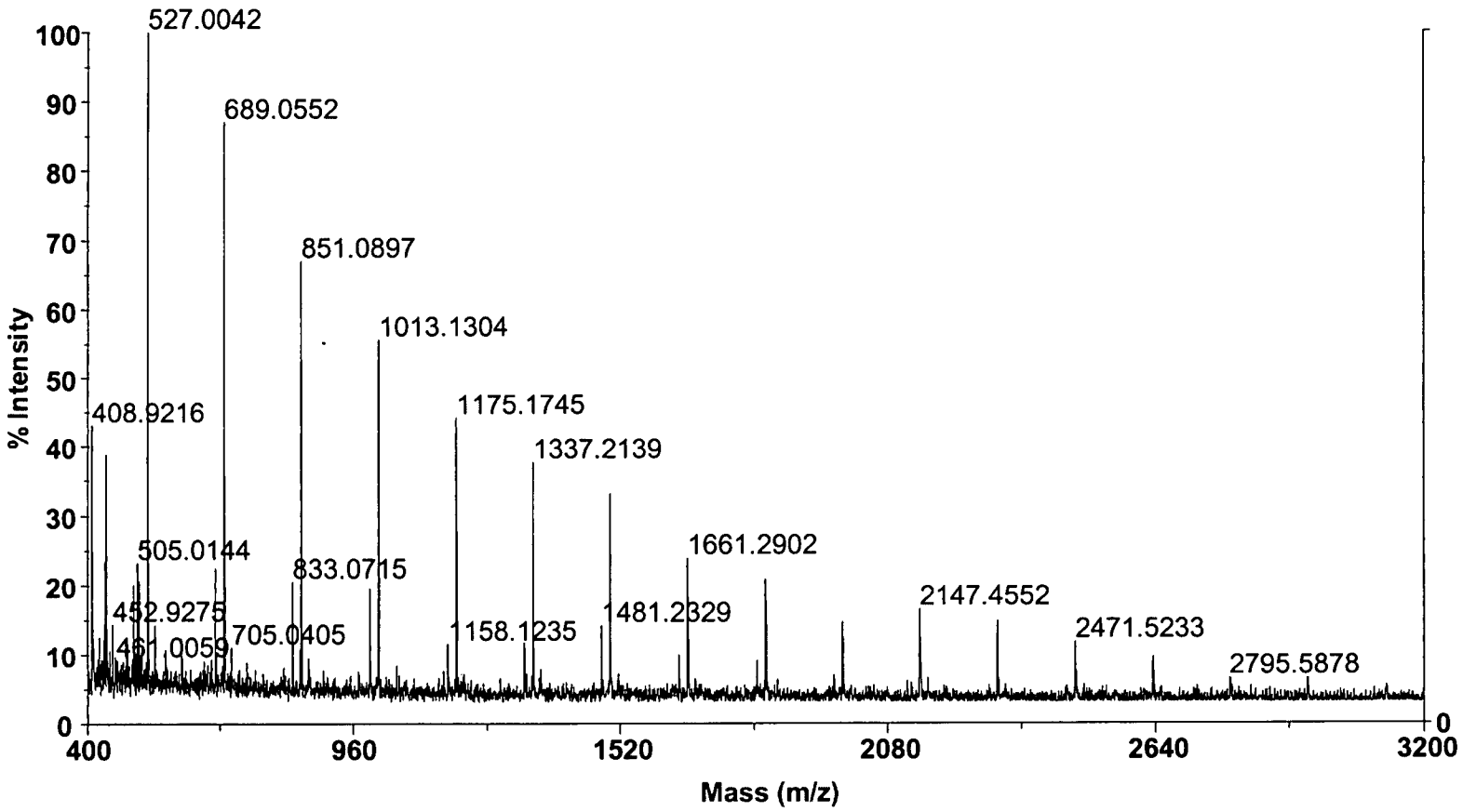


FIG. 43

Branched fructan reaction #3

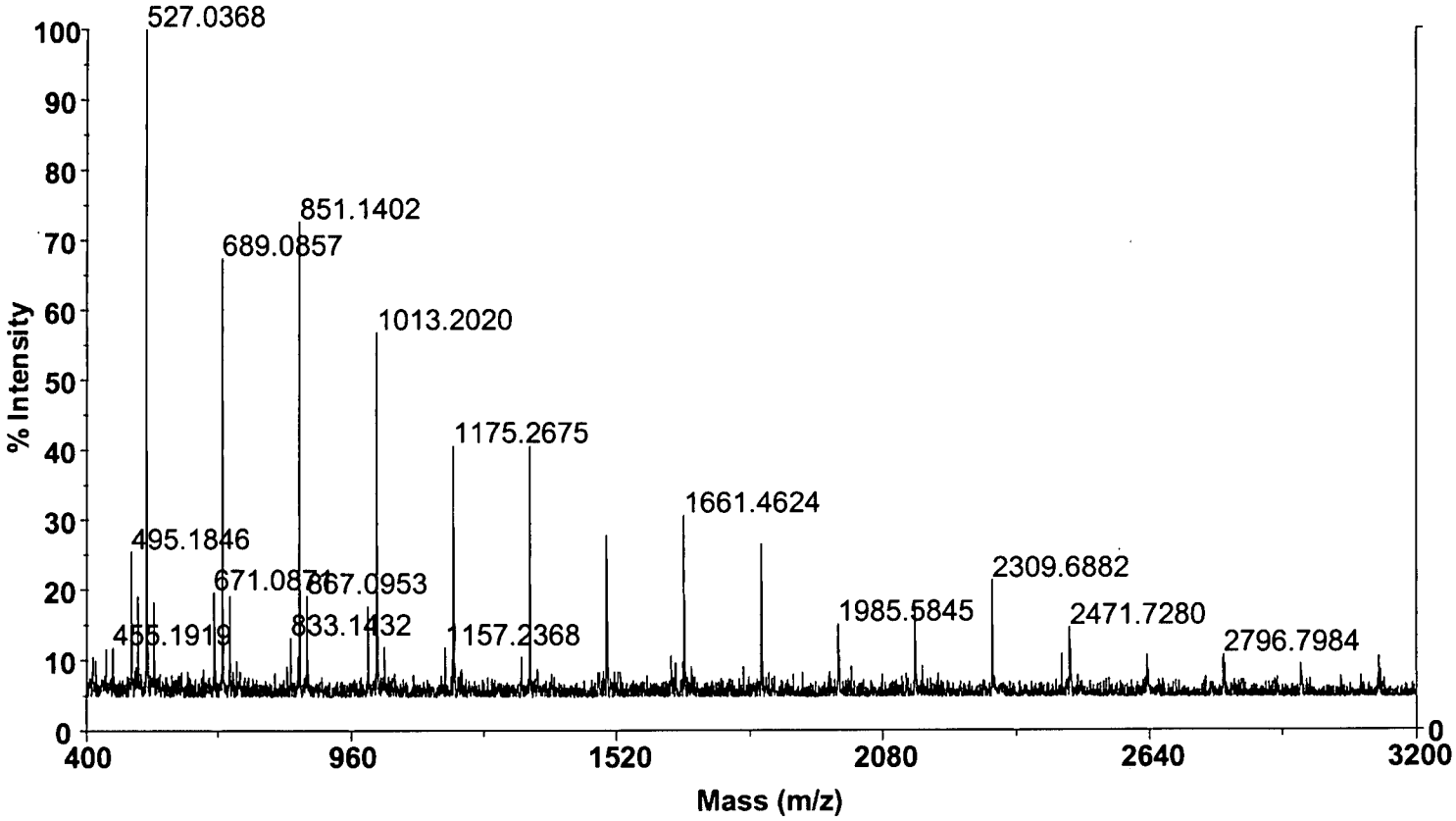


FIG. 44